

Original Research Article

Protective Effect of (-)-Epigallocatechin Gallate against Photo-Damage Induced by Ultraviolet A in Human Skin Fibroblasts

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Abstract

Purpose: To investigate the photoprotective effect of (-)-epigallocatechin gallate (EGCG), one of tea catechins, on human skin fibroblast (HSF) irradiated by ultraviolet A.

Methods: HSF cells were incubated in serum-free Dulbecco's Modified Eagle's Medium (DMEM) with or without EGCG for 2 h, and then irradiated by UV A. Blank (control) was incubated in DMEM without EGCG and UV A-irradiation. Cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Protein concentration of the samples was determined using a PA102 Bradford protein assay kit. Malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide anion radicals were determined using MDA assay kit, GSH-Px assay kit and superoxide anion radical assay kit, respectively.

Results: HSF viability decreased with dosage of UV A irradiation with 50 % lethal dose (LD₅₀) of 9 J/cm². Pre-incubation of HSF in 10 µg/mL EGCG aqueous solution for 2 h before exposure to UV A alleviated the suppressive effect of UV A on HSF. Compared to UVA irradiation alone, HSF viability and GSH-Px activity in the EGCG pretreatment increased by 18.3 and 103.4 %, accompanying decrease in level of superoxide anion radicals and MDA by 44.6 and 16.6 %, respectively.

Conclusion: EGCG alleviates UV A-induced HSF photo-damage through relieving oxidative stress by increasing activity of GSH-Px and scavenging capacity of superoxide anion radical.

Keywords: Irradiation, Catechins, Photoaging, Photoprotection, Malondialdehyde, Glutathione peroxidase, Superoxide anion radical

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INTRODUCTION

Human skin ageing is accelerated under external stress conditions such as ultraviolet (UV) irradiation and genotoxic agents. UV is a major environmental source of damage to the skin. The effect of UV on skin biology and immune system plays a major role in photoaging, inflammation and carcinogenesis [1]. Based on wavelength,

solar UV can be divided into UV A (320–400 nm), UV B (280–320 nm) and UV C (< 280 nm). High energy short wavelength UV C is absorbed by the ozone layer and atmosphere, therefore it does not reach the Earth's surface. UV B can partially penetrate the ozone layer and it makes up 5-10 % of atmospheric UV [2]. UV B has indirect detrimental effects on the immune system of the skin, oxidative stress responses,

and photoaging [3]. UV A radiation is more than 90 % of atmospheric UV irradiation [2], and it penetrates much deeper into the epidermis and dermis of the skin [4,5].

UV A radiation is absorbed by DNA and reacts with non-DNA chromophores, leading to formation of reactive oxygen species (ROS) which damage DNA, proteins, and lipids in the skin [4,5]. UV A plays a significant role in producing genotoxic bipyrimidine photo-products [6]. *In vivo* human studies show that UV A irradiation also has immunosuppressive effects [7]. Chronic and excessive UV exposure depletes human cutaneous defense mechanisms [8]. Human exposure to UV A has become a public health concern [9].

Experiments *in vivo* and *in vitro* show that plant polyphenols enhance intracellular antioxidant defense and anti-inflammatory capacity, therefore they can be used as photo-protective and chemo-preventative agents [4,10,11]. Tea is an important dietary source of plant polyphenols in which (-)-epigallocatechin-3-gallate (EGCG) is the most abundant one (Figure 1). Green tea polyphenols have been found to be effective at alleviating UV B induced damages [12-15]. However, their ability to reduce UV-induced photodamage in humans *in vivo* has not been addressed [16], and the effect of EGCG on mutation and ageing of cultured human skin fibroblast (HSF) caused by UV A irradiation and its underlying mechanism remain unclear [17]. This study was undertaken to determine whether EGCG was able to limit UVA-induced photodamage in HSF cells.

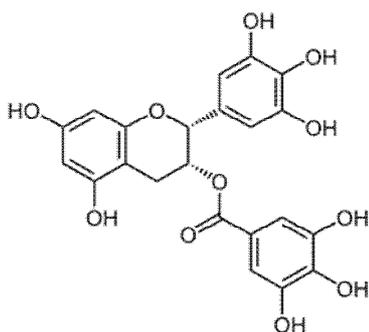


Figure 1: Molecular structure of (-)-epigallocatechin gallate (EGCG)

EXPERIMENTAL

Materials

The cell line used in the test was human skin fibroblast (HSF) purchased from the Cell Bank of

Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific Inc. (New Hampshire, USA). EGCG (95 % purity) was supplied by CinoTea Ltd (Hangzhou, China). The other chemicals used were AR grade and were purchased from Sinopharm Group Corporation (Beijing, China).

Cell culture and treatments

HSF cells were cultured in 10 cm culture dish in DMEM supplemented with 10 % FBS, penicillin (100 IU/mL) and streptomycin (100 µg/mL), at 37 °C in a humidified atmosphere containing 5 % CO₂. When confluence was attained at 80 %, the medium was disposed of, and the cells washed in phosphate buffered saline (PBS). The cells were then treated with EGCG aqueous solutions at concentrations 0, 10, 20 and 40 µg/mL for 2 h, and then irradiated by UV A using UV A lamps (Spectronics Corp. New York, USA) at dosage of 0, 4.5, 9.0 and 13.5 J/cm², respectively. A blank control was incubated in serum-free medium without EGCG or UV A-irradiation.

Cell viability assay

The cell viability was determined by the ability of mitochondria to convert 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to formazan dye. The MTT was dissolved at a concentration of 5 mg/mL in PBS. The cells to be tested were cultured overnight onto 96-well plate at a density of 1×10^5 cells/well. 20 µL of MTT solution was added to each culture well, and then the plates were incubated at 37 °C in an atmosphere of 5 % CO₂ for 4 h. The medium was replaced by dimethyl sulfoxide (DMSO) (200 µL per well), pipetting up and down until the crystals being dissolved. Absorbance of the samples was measured at 492 nm in an ELISA reader (Thermo Fischer Scientific, MA, USA). The results of cell viability were presented as the percentage of 492 nm absorbance of tested samples to that of the control.

Biochemical assays

Malondialdehyde (MDA) and superoxide anion radical were determined using MDA assay kit and superoxide anion radical assay kit (Jiancheng Sci-Tech Co. Ltd., Nanjing, China), respectively, according the manufacturer's guides. Activity of glutathione peroxidase (GSH-Px) was determined using GSH-Px assay kit (Jiancheng Sci-Tech Co. Ltd., Nanjing, China) following the kit instruction and expressed as enzyme active unit per gramme of protein. The

protein was tested using a PA102 Bradford protein assay kit [Tiangen Biotech (Beijing) Co., Ltd., Beijing, China] according to instructions of the kit.

Statistical analysis

Data were analyzed using Student's t-test and presented as mean \pm standard deviation (SD). $P < 0.05$ was regarded as statistically significant.

RESULTS

Effect of UV A and EGCG on viability of HSF cells

Viability of HSF cells was suppressed by UV A irradiation in a dose dependent manner (Figure 2). The lethal dosage 50 % (LD50) at which viability of HSF cells decreased by 50 % was about 9 J/cm². At the LD50, the HSF cells were seriously injured by UV A irradiation, but the cell viability could be partially recovered by UV protection armamentarium. Therefore, UV A irradiation dosage 9 J/cm² was used in the following experiments to test the effects of EGCG on UV A induced HSF damage. If the HSF cells were pre-incubated in EGCG aqueous solution for 2 h before exposed to 9 J/cm² UVA irradiation, the suppressive effect of UVA on the HSF was relieved (Figure 3). However, there was no significant difference in growth rate as the EGCG concentration increased from 10 μ g/mL to 40 μ g/mL (Figure 3). Based on this result, EGCG concentration 10 μ g/mL was used in the subsequent tests.

The HSF cells were incubated in EGCG aqueous solution for 2h before irradiated at 9 J/cm² UVA.

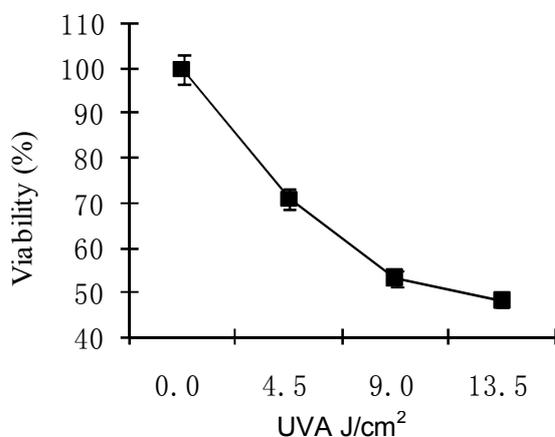


Figure 2: Effect of UVA irradiation on viability HSF cells

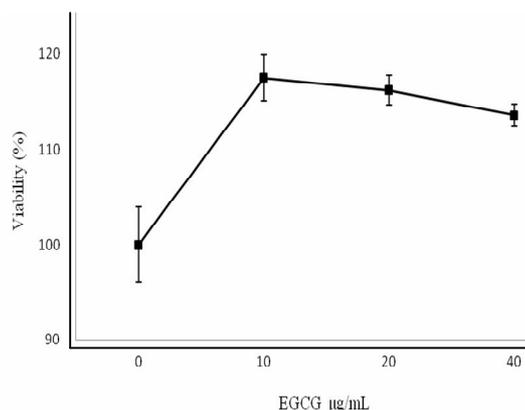


Figure 3: Effect of EGCG on viability of HSF cells exposed to UVA

Effect of EGCG on some oxidative stress parameters of HSF cells exposed to UV A

UV A irradiation (9 J/cm²) significantly increased MDA level in the HSF cells. However, the UVA-induced increase in MDA level was suppressed by pre-treatment with EGCG (10 μ g/mL) before exposure to UVA irradiation (UV A + EGCG). Figure 4 showed that MDA level of treatment (UV A + EGCG) was 16.6 % lower than UV A irradiation alone (UV A). MDA is an end product of lipid peroxidation. This suggests that EGCG has anti-lipid peroxidation activity, which results in decrease in MDA accumulation.

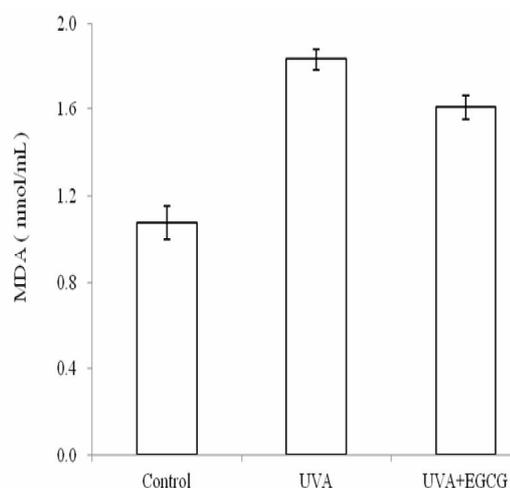


Figure 4: Effect of EGCG on MDA level in HSF cells exposed to UV A

Figure 5 shows that superoxide anion level increased when the HSF cells were exposed to 9 J/cm² UV A. However, the increase was suppressed as the cells were pre-incubated with EGCG (10 μ g/mL) before exposed to UV A. The responses of GSH-Px to UV A irradiation and EGCG pre-treatment showed a reverse trend as

the MDA level and the superoxide anion level (Figures 4-6).

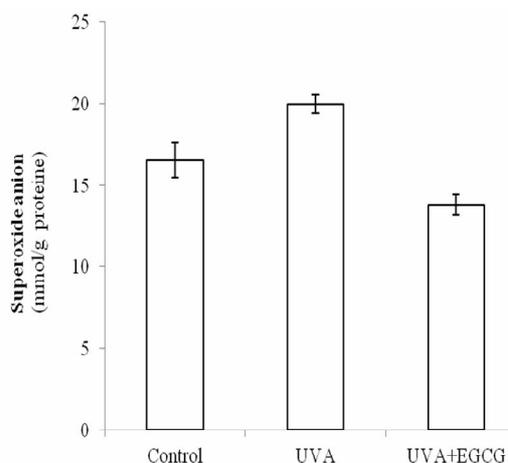


Figure 5: Effect of EGCG on superoxide anion level in HSF cells exposed to UVA

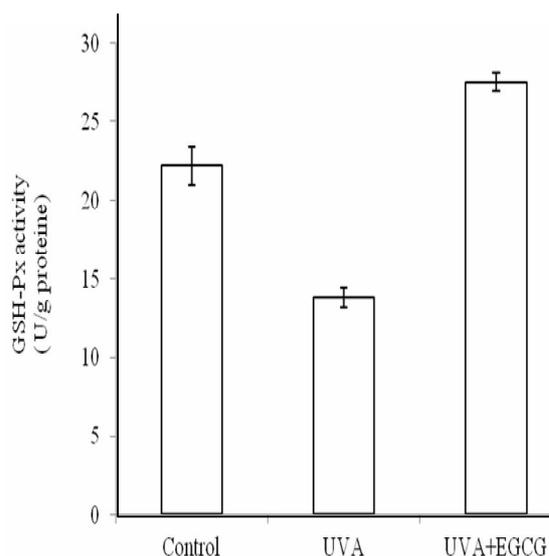


Figure 6: Effect of EGCG on GSH-Px activity in HSF cells exposed to UVA

DISCUSSION

UV radiations absorbed by the skin surface can produce harmful compounds called free radicals or reactive oxygen species (ROS), which are chemically reactive molecules containing oxygen such as oxygen ions and peroxides and can cause skin cancer and premature aging [18]. The underlying mechanism of UV radiation damage is known to be the generation of ROS resulting from lipid peroxidation of the membranes in the skin cells [19-22]. Lipid peroxidation is one of the sequels of superoxide anion generation [23]. One possible method to prevent skin from UV damage is to use a powerful antioxidant to quench the generated ROS [24]. The results of

this study showed that EGCG suppressed the increase in levels of superoxide anion and MDA of the HSF cells exposed to UV A (Figures 4-5), resulting in the increase of HSF viability (Figure 3). MDA is an end product of lipid peroxidation [23]. These suggest that EGCG, a potent free radical scavenger, is able to curtail the damaging effects of UV A by inhibiting the generation of superoxide anions and lipid peroxidation.

Glutathione peroxidase (GSH-Px) is an enzyme family with peroxidase activity whose biochemical function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water so as to protect the organism from oxidative damage. An investigation on the skin fibroblasts from subjects of different ages showed that GSH-Px activity in the skin fibroblasts decreased with age, accompanying an increase in MDA level [20]. Hydrogen molecule (H_2) has been known as a safe antioxidant in the prevention and therapeutic approach towards several diseases. Bathing with hydrogen reduced water (HRW) before exposure to UV B significantly reduced the levels of skin damage induced by the UV B, accompanying significant increase in activity of GSH-Px [22]. Keratinocyte-specific knockout mice lacking the glutathione peroxidase 4 (GSH-Px 4), an isoenzyme of GSH-Px, increased lipid peroxidation in cultured keratinocytes and whole skin [25]. Loss of GSH-Px 4 in the epidermis caused epidermal hyperplasia, dermal inflammatory infiltrate, dysmorphic hair follicles, and alopecia in perinatal mice [25]. The compound 6-CySeCD, a GSH-Px mimic, could relieve the damage induced by UV B irradiation in HaCaT cells [26]. The present study showed that pre-treatment of EGCG alleviated the suppression of GSH-Px activity induced by UVA irradiation (Figure 6), which might be partially contributed to the lower levels of superoxide anion and MDA by reducing oxidative stress.

There was study showed that epicatechin (EC), one of green tea catechins, protected HSF cells against UVA-induced damages by its sunscreen and its effect on the synthesis of new proteins [27]. The antioxidative activity of flavonoids are considered to be depended on the number of OH group in their molecules. The more OH groups it has, the stronger its power to fight against UV stressed skin aging [28,29]. EGCG is the ester of epigallocatechin and gallic acid, and so its molecule has more OH groups than EC, thus making EGCG a stronger potential candidate as a dermatological protectant.

CONCLUSION

The present study confirmed that UV A irradiation suppressed viability of human skin fibroblast (HSF) and pre-treatment of HSF with 10 µg/mL EGCG for 2 h before exposure to UV A alleviated the suppressive effect of UVA. The underlying mechanism for EGCG protecting HSF cells against UVA-induced photo-damage is considered to be its relieving oxidative stress by suppressing the UVA-induced decrease in glutathione peroxidase (GSH-Px) activity and inhibiting the UVA-induced increase in superoxide anion radicals, lipid peroxidation and malondialdehyde (MDA).

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