Original Research Article

Protective effect of (-)-epigallocatechin gallate on ultraviolet b-induced skin damage in hairless mice

Chang Liu[1], Xin-Qiang Zheng[1], Li-Ping Xiang[2], Jian-Liang Lu[1], Curt Anthony Polito[1] and Yue-Rong Liang[1]*

[1]Zhejiang University Tea Research Institute, Hangzhou 310058, [2]Guizhou Tea and Tea Products Quality Supervision and Inspection Center, Zunyi 563100, PR China

*For correspondence: **Email:** yrliang@zju.edu.cn; **Tel/Fax:** +86 571 8898 2704

Abstract

Purpose: To investigate the protective effect of green tea (-)-epigallocatechin gallate (EGCg) on ultraviolet B (UV-B)-induced skin damages in hairless mice in order to develop a natural sunscreen compound for use in skin care products and cosmetics.
Methods: EGCg was dissolved in acetone at concentrations of 1.0, 10.0 and 50.0 mg/mL, and topically applied to the skin of hairless mice at doses of 0.2 mL/cm^2, with acetone as control. The mice were then irradiated by 100 µW/cm^2 UV-B for 30 min daily. EGCg treatment and UV-B irradiation were carried out daily for 28 consecutive days. The mice were then sacrificed and their dorsal skin examined by transmission electron microscopy (TEM) on the 28th day.
Results: UV-B irradiation induced severe macroscopic skin damage including chapping, cracking and abnormal desquamation in the treated hairless mice. EGCg showed dose-dependent protective effects against UV-B induced damage on the skin. Treatments with 10.0 and 50.0 mg/mL EGCg alleviated UV-B-induced skin damage by suppressing both keratinocyte apoptosis and mitochondrial dysfunction, along with inhibiting the production of melanin pigment.
Conclusion: Topical application of green tea EGCg shows dose-dependent protective effect against UV-B-induced damage on hairless mouse skin. Thus, the plant compound can potentially be used as an alternative agent for photoprotection against UV-B exposure.

Keywords: UV-B, Green tea EGCg, Photoprotection, Stratum corneum, Mitochondrion, Melanosome

INTRODUCTION

Skin is an organ of the integumentary system, made up of multiple layers of ectodermal tissue, which serves as a protective barrier over the body's surface. The occurrence of many skin diseases such as skin tumors and skin aging are considered to be related to excessive exposure to ultraviolet B (UV-B) light, usually from exposure to sunlight. UV-B is a form of electromagnetic radiation with a wavelength ranging from 280 nm to 320 nm [1]. UV-B triggers generation of cyclobutane pyrimidine dimers (CPDs) and some photoproducts, which cause DNA damage and mutation in the epidermal cells, resulting in carcinogenesis [2].

Although epidemiological studies claim that application of sun screen lotion is helpful for skin protection, sun screen lotion may be inadequate for preventing all UV related skin damages. Due to their antioxidant properties, botanical substances, such as (-)-epigallocatechin-3-gallate (EGCg) in green tea, are considered to be

promising natural materials for preventing skin from UV related damage and have attracted a lot of recent research attention. Phenolic compounds are reported to possess anti-radiation properties because of their antioxidant and anti-inflammatory properties [3].

EGCg, a major polyphenolic constituent in green tea, is an effective free radical scavenger [3]. EGCg has been shown to inhibit UV-A-induced melanogenesis via suppression of both tyrosinase (TRY) activity and melanosome maturation, and it is an effective melanogenesis inhibitor [4]. Topical application of EGCg in hydrophilic cream has also showed high protection of photocarcinogenesis in UV-B-radiated SKH-1 hairless mouse model [5]. There are results from clinical studies showing that EGCg treatment reduced the occurrence of tumors in different organs including skin, liver, lungs, breast, stomach and prostate [6]. A formulation containing tannase-converted green tea extract is effective in inhibiting UV-B-induced oxidative damage and in protecting skin from UV-B photodamage [7].

Previous studies involving mechanisms of EGCg acting on UV-B induced damages were usually investigated in cell culture systems. Tests using HaCaT cells concluded that green tea polyphenols afforded protection against UV-B induced stress by interacting with UV-B induced reactive oxygen species (ROS) and attenuating mitochondrion-mediated apoptosis [8]. However, these mechanisms need to be evaluated and verified in animal models and/or clinical studies [7,9].

The present study was designed to investigate the photoprotective effect of EGCg on UV-B-induced skin damages in hairless mice by using acetone as a vehicle solvent for delivering EGCg. Ultrastructural changes in the skin tissues induced by UV-B were observed by transmission electron microscopy (TEM).

EXPERIMENTAL

Animals

Forty-two hairless mice (21 males and 21 females) were purchased from the Shanghai Laboratory Animal Center, Chinese Academy Sciences (Shanghai, China). The mice were housed in an animal facility and acclimatized for one week under conditions at 27 ± 1 °C, 35 ± 5 % humidity, 12 h light/12 h dark cycle, free access to water and a normal caloric standard diet.

The experimental procedures involving animals and their care were conducted in conformity with the Guide for Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH publication no. 85 - 23, 1996). Ethical approval for the animal studies was issued by the Ethics Committee for Animal Experimentation of the Shanghai Laboratory Animal Center, Chinese Academy Sciences (Shanghai, China), with the approval ref. no. SCXK (Hu)-2012-0005.

Treatment

Before beginning the study, the mice were randomly divided into 6 groups: Blank Control (BC, with no EGCg or UV-B irradiation treatments); Negative Control (NC, receiving topical application of vehicle solvent (acetone) and UV-B irradiation); Positive Control (PC, receiving topical application of KIEHL'S ultra light daily UV defense (SPF50 PA+++) and UV-B irradiation); Low Level EGCg Treatment (LL, receiving topical application of 1.0 mg/mL EGCg dissolved in acetone and UV-B irradiation); Medium Level EGCg Treatment (ML, receiving topical application of 10.0 mg/mL EGCg dissolved in acetone and UV-B irradiation); and High Level EGCg Treatment (HL, receiving topical application of 50.0 mg/mL EGCg dissolved in acetone and UV-B irradiation).

For each EGCg treatment, the EGCg acetone solutions were topically applied to the skin of the hairless mice at measured dosages of 10 $\mu L/cm^2$. 30 min later, the mice were exposed to 30 min of UV-B irradiation (100 $\mu W/cm^2$). UV-B radiation was supplied by six (6) UV-B lamps (Spectronics BLE-1T158 Tube, 15 watt). Each EGCg treatment and UV-B irradiation were carried out daily for consecutive 28 days. Then, on the 28th day, the mice were killed and their dorsal skins were sampled for transmission electronic microscopy (TEM) examination.

Transmission electron microscopy (TEM)

Sample fixation

The skin tissue samples were preliminarily fixed with 2.5 % (w/v) glutaraldehyde in phosphate buffer (pH 7.0) for 4 h, and then washed three times using phosphate buffer (pH 7.0). The preliminary fixed samples were finally fixed in 1 %(w/v) OsO_4 dissolved in phosphate buffer (pH 7.0) for 1 h and then washed three times in phosphate buffer (pH 7.0).

RESULTS

Effect of EGCg on morphological characteristics of stratum corneum

Over the course of the UV-B treatments, the dorsal skins of the hairless mice were affected, wrinkling after the first week, peeling and finally signs of spiloplaxia. Moreover, there were obvious differences in morphological characteristics of stratum corneum among the six treatment groups (Figure 1). In the negative control group, UV-B exposure induced severe macroscopic skin damage such as chapping, cracking and abnormal desquamation (Figure 1, NC). Topical application of UV defense agent (KIEHL'S ultra light daily UV defense (SPF50 PA+++)) showed good protective effect on the hairless mice skin exposed to UV-B irradiation (Figure 1, PC).

The protective effects of EGCg was dose-dependent. Low level EGCG treatment (1.0 mg/mL) showed little protective effect on the skin (Fig 1, LL). However, treatments of medium (10.0 mg/mL) and high (50.0 mg/mL) levels of EGCg produced protective effects similar to that of the positive control group (Figure 1, PC, ML and HL). These results suggest that EGCg produces protective effects on the skins of hairless mice exposed to UV-B radiation in a dose dependent manner.

Effect of EGCg on ultra-structure of epidermis

Differences in the thickness of the stratum corneum layer of the skins were recognized based on the type of treatment (Figure 2, Table 1).

Dehydration

The fixed skin samples were dehydrated in an increasing concentration series of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, 95 % and 100 %, v/v), lasting for 20 min at each concentration level.

Infiltration

The dehydrated skin samples were placed in a mixture of acetone and Spurr resin ERL-4206 (1:1) for 1 h at room temperature, then were transferred into a mixture of acetone and Spurr resin ERL-4206 (1:3) for 3 h, and finally transferred into pure Spurr resin overnight.

Embedding and ultrathin sectioning

The above fixed skin samples were placed in capsules containing embedding medium (Spurr resin ERL-4206) at 70 °C for 9 h. Sections with thickness 70 – 90 nm were made on a Reichert-Jung OM U2 ultra-microtome (Reichert-Jung Co., Heidelburg, Germany) and were stained using uranyl acetate and alkaline lead citrate for 15 min [8]. The sections were examined and photographed with a Hitachi transmission electronic microscope (Hitachi Model H-7650, Hitachi Ltd., Tokyo, Japan).

Statistical analysis

One-way analysis of variance followed by Tukey's multiple comparisons test was performed using the scientific statistic software SPSS (version 20.0). $P = 0.05$ was considered statistically significant for differences between treatments.

Figure 1: Effect of UV-B and EGCg on skin aging of hairless mice. BC: blank control, neither EGCg nor UV-B irradiation; PC: positive control, receiving topical application of KIEHL'S ultra light daily UV defense (SPF50 PA+++) and UV-B irradiation; NC: negative control, receiving topical application of vehicle solvent (acetone) and UV-B irradiation; LL: low level EGCg treatment, receiving topical application of 1.0 mg/mL EGCg dissolved in acetone and UV-B irradiation; ML: medium level EGCg treatment, receiving topical application of 10.0 mg/mL EGCg dissolved in acetone and UV-B irradiation; HL: high level EGCg treatment, receiving topical application of 50.0 mg/mL EGCg dissolved in acetone and UV-B irradiation

After 28 days of UV-B irradiation, the stratum corneum thickness was increased most in the skins of the negative control group while increasing least in the positive and high level EGCg treatment groups. The positive control group was most effective at preventing stratum corneum incrassation. Low level EGCg (1.0 mg/mL) had no protective effect on the statum corneum, while medium and high levels of EGCg (10.0 and 50.0 mg/ mL) significantly inhibited the stratum corneum incrassation induced by UV-B irradiation (Table 1).

In the blank control without UV-B irradiation, few melanosomes, in which pigment melanin precipitated, were observed (Figure 2, CB). However, there were many melanosomes in the UV-B irradiated treatments (Figure 2, NC, PC, LL, ML, HL). These results suggest that UV-B stimulated the production of pigment melanin. The positive control with UV defense agent and treatment with high level EGCg (HL) had less melanosomes than the negative control (NC) and

treatments with low level (LL) and high level (HL) EGCg (Figure 2), suggesting that high level EGCg and the UV defense agent suppressed the production of pigment melanin induced by UV-B irradiation.

Figure 3 shows that obvious degenerations were observed in mitochondria of negative control (Figure 3, NC) and low level EGCg (1 mg/ml) (Figure 3, LL), in which mitochondrial crest and matrix density were reduced, and vacuolation was observed. However, there were no significant differences in mitochondrial structures between treatments of medium and high levels of EGCg (Figure 3, ML & HL), blank control (Figure 3, BC) and positive control (Figure3, PC), in which mitochondrial membrane and mitochondrial matrix were clearly observed. These results indicate that an appropriate concentration of EGCg protected skin damages in hairless mice irradiated by UV-B through its protective effect on mitochondria.

Table 1: Stratum corneum thickness of various treatments [a] (mean ± SD, μm [b])

BC	NC	PC	LL	ML	HL
	4.65±0.10A	20.88±3.48B	21.38±0.22C	12.62±0.12D	4.85±0.31A
4.27±0.07A					

[a]: The group labels are the same as in Figure 1; [b]: Mean values followed different capital letters were significantly different at $p < 0.05$
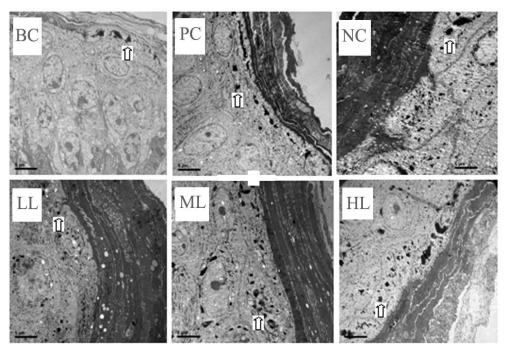

Figure 2: Effect of UV-B and EGCg on ultra-structure of epidermis. The group labels are the same as in Figure 1; arrows denote melanosomes
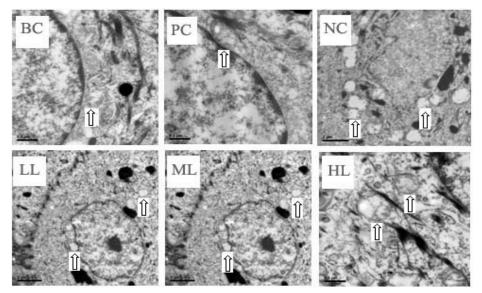
Figure 3: Effect of UV-B and EGCg on mitochondria. The group labels are the same as in Figure 1; arrows denote mitochondria

DISCUSSION

Cutaneous aging is a complex biological phenomenon commonly embodied in wrinkling, sagging and increased laxity. Although intrinsic skin aging is considered to be genetically determined, cumulative exposure to sunlight contributes to and exacerbates a majority of the skin's 'aging' [10]. For example, skin exposed to sunlight or UV rays becomes rough with wrinkles and irregular pigmentation and morphological characteristics. An increase in matrix metalloproteinase (MMP) activity and a decrease in expression of collagen type I protein are considered to be responses of skin to UV stress, which leads to massive elastosis and collagen degeneration [11]. Research suggests that MMP-1 (one of MMP enzymes) is the major enzyme responsible for digestion of collagen type I protein in UV-irradiated skin [12]. Tissue inhibitor of metalloproteinase (TIMPs) and inhibitors of MMPs play a major role in the maintenance of extracellular matrix homeostasis [13]. Sen *et al* treated MDA-MB-231 cells with 20 µmol/L EGCg for 48 h, and the expression of TIMP-1 increased 32-fold compared to the untreated control set, which indicates that EGCg enhanced the expression of TIMP-1, along with the reduction in mRNA expression and activity of MMP-9 [14]. The present study showed that EGCg had protective effects on the UV-B damaged skins of hairless mice. It can be concluded that EGCg stimulated the expression of TIMP-1 and inhibited the expression of MMP-1, which played a role in the maintenance of extracellular matrix homeostasis.

The present TEM study revealed that medium and high levels of EGCg (10 and 50 mg/mL) significantly inhibited the incrassation of stratum corneum induced by UV-B irradiation. Corneocytes, composing most of the stratum corneum, are terminally differentiated keratinocytes. Intensive irradiation of UV-B injured the keratinocytes (which form the proliferative layer of the epidermis) resulting in an obvious increase in the amount of dead keratinocytes and thickening of the stratum corneum [15]. The intercellular lipids in stratum corneum play an important role in maintaining the barrier properties of the skin. The increase of stratum corneum lipids, along with the decrease in epidermal permeability, was a response of the epidermis to UV-B irradiation [16].

Mitochondrion, one of the most prominent and important organelles in eukaryotic cells, provides energy from metabolic fuels through oxidative phosphorylation [17]. It is the cell's powerhouse. In response to UV-B irradiation, there was a marked increase in the number of melanosomes [1]. The present study showed that EGCg suppressed the formation of melanosomes in a dose-dependent manner, suggesting that high levels of EGCg alleviated skin browning induced by UV-B. EGCg tea catechins exhibited sunscreen properties that alleviated UV-B stress [18]. It is clear that melanogenesis induced by UV radiation is characterized by proliferation and differentiation of functioning melanocytes. There is research indicating that tyrosine-kinase p59Fyn is a crucial factor influencing dedifferentiation and transformation of melanocytes. The expression of tyrosine-kinase

P59Fyn leads to down-regulation of mitogen-activated protein kinase phosphatase 1 (MKP-1) and suppression of the differentiation of melanocytes [19]. There was also an *in vitro* study which indicated that EGCg inhibited the activity of tyrosine-kinase P59Fyn, resulting in suppression of melanocyte cell transformation [20]. The tyrosine-kinase p59Fyn is considered to be a target of EGCg [19]. The suppressive effects of EGCg on the formation of melanosomes is thought to be related to its regulation effect on the expression of tyrosine-kinase p59Fyn.

It has been reported that UV-B irradiation altered the structures and functions of mitochondria. Excessive exposure to UV-B induced formation of reactive oxygen species (ROS), which led to changes in mitochondrial size and internal cristae (also known as mitochondrial-mediated apoptosis) [8]. It is hypothesized that ROS activates permeability transition pore opening, leading to mitochondrial swelling. This in turn induces the release of cytochrome c from cardiolipin and outer membrane rupture via proapoptotic protein translocation [21]. The present TEM study showed that medium and high levels of EGCg prevented mitochondria from vacuolization and degeneration induced by UV-B irradiation. It is considered that the EGCg afforded protection against the UVB-induced stress via both interacting with UVB-induced reactive oxygen species and attenuating mitochondrion-mediated apoptosis.

CONCLUSION

The findings of this present study show that topical application of green tea EGCg protects the skin of hairless mice from UV-B-induced damages in two ways, viz, by suppressing both mitochondrial dysfunction, and by inhibiting keratinocyte apoptosis. Therefore, EGCg is a potential alternative agent for photoprotection against UV-B irradiation.

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DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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