

Photoprotective Effect of Topical EGb 761 and Korean Red Ginseng in C57BL/6 Mice

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Abstract

Exposure to UVB radiation can cause diverse biological photodamage to skin. EGb 761 and Korean red ginseng are the major and most effective natural drug against a variety of oxidative damage. But, the protective effects against UVB radiation have not been clearly identified. In this study, we investigated the protective effect of topical EGb 761 and Korean red ginseng on pigmentation by UVB radiation. Pro-inflammatory cytokines(IL-1 β , IL-6 and TNF- α) and melanogenesis proteins(tyrosinase, TRP-1 and TRP-2) mRNA were measured by reverse transcription-polymerase chain reaction(RT-PCR) analysis. The in vivo protection against pigmentation was calculated using chromameter. The mRNA level of IL-1 β and TNF- α were increased by UVB irradiation in treated and non-treated group, while no significant changes were observed in IL-6 level. Topical treatment with EGb 761 and Korean red ginseng remarkably reduced expression of tyrosinase, TRP-1 and TRP-2 in the non-irradiated and irradiated skin. Application of EGb 761 and Korean red ginseng significantly protected the UVB-induced skin pigmentation and Korean red ginseng was more effective. Our study suggests that topical EGb 761 and Korean red ginseng can regulate melanogenic proteins and protect UVB radiation on skin pigmentation.

Introduction

Exposure of the skin to ultraviolet B radiation induced skin pigmentation. In the process of UVB-induced pigmentation, an increase in melanin production is an important event, which is a defense of skin against the harmful effects of UV due to its ability to absorb and reflect UV energy. From this protective point of view, there has been reported on various natural compounds targeted at regulating melanin production. Melanogenesis is regulated by melanogen, inflammatory factors and melanogenic proteins, which are all released from cells surrounding melanocytes in response to UVB-stimulation. EGb 761 and Korean red ginseng is the most effective antioxidant and can offer protection against a variety of oxidative damage and the

induction of epidermal lipid peroxidation. But, the protective effects of EGb 761 and Korean red ginseng on skin damaged UV have not been clearly reported. Therefore, this study was carried out to investigate the protective effect of EGb 761 and Korean red ginseng against pigmentation induced by UVB based on mRNA level and visual assessment. To observe the protective effect of compounds on melanin production, we induced activation of pro-inflammatory cytokine(IL-1 β , IL-6 and TNF- α) and melanogenic proteins(tyrosinase, TRP-1 and TRP-2) by UVB irradiation. The in vivo protection by compounds against pigmentation was performed to assess the depigmenting effect using chromameter.

Materials and Method

Animals and treatment

Female C57BL/6 mice(8 weeks old) were used. The dorsal skin of mice was shaved with clipper one day before the start of the experiment. EGb 761 extract(Yuyu industrial co. Ltd.), Korean red ginseng (Cheong-Kwan-Jang, Korea Ginseng corp.) were dissolved in a PEG lotion at a concentration of 1% (w/v) and 200ul of each lotions was applied to the shaved dorsal skin. Topical treatment was applied for 5days before UVB irradiation.

UVB-induced expression of pro-inflammatory cytokine and melanogenesis proteins in tissue

The mice were divided into six groups and each group was eight mice. The shaved skin was exposed to 1MED(1.4KJ/m²) UVB radiation for 6 days. The irradiated skin was treated immediately after irradiation with lotion containing EGb 761 extract and Korean red ginseng extract. The protection by each extract was studied using RT-PCR analysis in RNA prepared from dorsal skin tissue. RT-PCR was undertaken according to the following cycles, beginning at 42 for 1h, 94 $^{\circ}$ C for 5min, followed by 31 cycles of 95 $^{\circ}$ C for 5min; 95 $^{\circ}$ C for 1min; 60 $^{\circ}$ C for 1min; 72 $^{\circ}$ C for 1min, ending of 72 $^{\circ}$ C for 10min. The amplified products were electrophoresed 1.2% agarose gel with 1 μ g/ml of ethidium bromide in TBE buffer and visualized by the UV transilluminator.

UVB-induced pigmentation in mice

The dorsal of each mice were exposed to 1MED UVB radiation. For 12 days. The irradiated skin was treated immediately after irradiation with lotion containing each extract. The degree of pigmentation was assessed as the L-value measured using a chromameter(Spectron Tech color measurement system)

Results and Discussion

Effect of EGb 761 and Korean red ginseng on the expression of pro-inflammatory cytokine after UVB exposure

The ability of EGb 761 and Korean red ginseng to inhibit UVB-induced IL-1 β , IL-6 and TNF- α gene expression was determined by RT-PCR analysis. Topical treatment with EGb 761 significantly increased the levels of IL-1 β and IL-6 gene expression in the non-irradiated skin(Fig 1.) The mRNA levels of IL-1 β and TNF- α gene expression increased by UVB

exposure. By contrast, levels of IL-6 gene expression remained unchanged following UVB irradiation. Ultraviolet B radiation of skin causes abundant release of numerous cytokines including IL-1 β , IL-6 and TNF- α . Release of IL-1 β , IL-6 and TNF- α decreases melanin production and induces post-inflammatory hypopigmentation. According to our result, EGb 761 and Korean red ginseng play a role in regulating these cytokines mediated UVB irradiation.

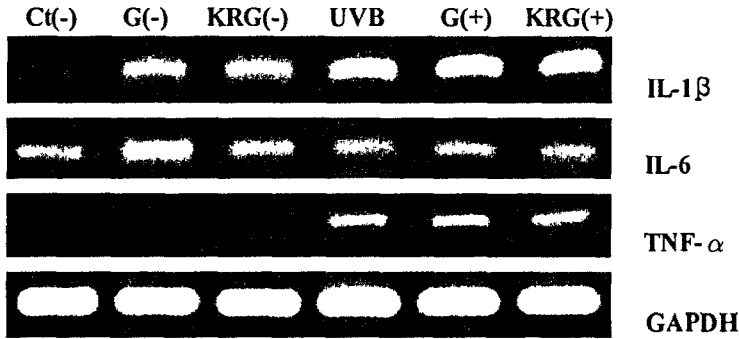


Fig. 1. Cytokine mRNA expression in C57BL/6 mice. Ct(non-UVB), G(treated 1% EGb 761), KRG(treated 1% Korean Red ginseng) (-); non-exposed UVB, (+); exposed-UVB

Effect of EGb 761 and Korean red ginseng on the expression of melanogenic proteins after UVB exposure
 For proper melanin production, several specific enzymes such as tyrosinase, two tyrosinase-related proteins(TRPs) are known, TRP-1 and TRP-2 are required. To study the protection level of EGb 761 and Korean red ginseng extract against UVB radiation, the expression of tyrosinase, TRP-1 and TRP-2 in UVB-irradiated mice skin was examined by RT-PCR analysis. Topical treatment with EGb 761 and Korean red ginseng extract remarkably reduced expression of tyrosinase, TRP-1 and TRP-2 in the non-irradiated mice(Fig 2.) Their expression were increased after exposure to UVB, whereas the expression in topical treatment was significantly inhibited. These results show that topical EGb 761 and Korean red ginseng may contribute to the regulation of melanogenic proteins.

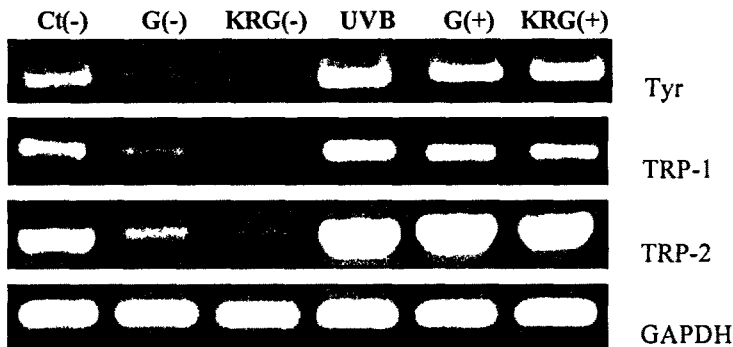


Fig. 2. mRNA expression of melanocyte specific proteins in C57BL/6 mice. Ct(non-UVB), G(treated 1% EGb 761), KRG(treated 1% Korean Red ginseng) (-); non-exposed UVB, (+); exposed-UVB

Depigmenting effects on C57BL/6 mice skin

The levels of protection by EGb 761 and Korean red ginseng extract against pigmentation was examined by the degree of pigmentation decrease compared to the vehicle group. There was a clear distinction UVB-irradiation group and non-irradiation group(Fig 3.) Topical application of 1% each extract group significantly decreased the UVB-induced pigmentation and Korean red ginseng extract had more protective effect.

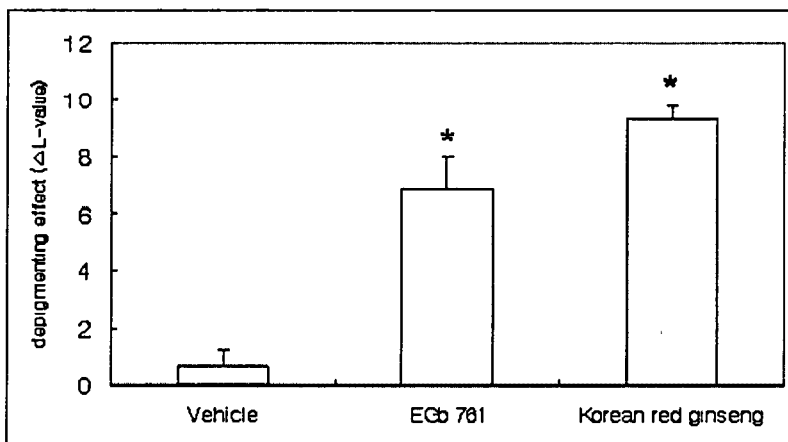


Fig. 3. The degree of pigmentation decrease(ΔL -value) control and compounds(1% EGb 761, Korean red ginseng). The data are expressed as a mean ΔL -value \pm SEM, t-test was used for the statistical analysis of the data($p < 0.05$ vs.control). groups of eight mice were used in this experiment.

Conclusion

Ultraviolet B irradiation increased the mRNA level of pro-inflammatory cytokines, melanogenic proteins and induced skin pigmentation in C57BL/6 mice. Topical application of EGb 761 and Korean red ginseng can modulate in both irradiated skin and non-irradiated skin. Skin pigmentation-induced UVB radiation decreased by application of EGb 761 and Korean red ginseng. In conclusion, EGb 761 and Korean red ginseng were protective effect against UVB radiation. This study reveals that EGb 761 and Korean red ginseng may play an important role in protecting UVB on skin pigmentation and have an effectiveness of whitening agents.

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