Ultraviolet B irradiation induces barrier dysfunction in epidermal keratinocytes, which can be repaired by ethanol extract of peanut sprouts (EPS), a new antioxidant to be originated from peanuts

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Introduction and Methods

◯ Accumulation of studies have shown that barrier dysfunction in the skin is closely related with UVB-induced skin inflammation. In our previous study, we found that a natural product from peanut, named ethanol extract of peanut sprouts (EPS), was found to have a potent antioxidant activity by showing suppressive activities of the induced expression of COX-2 and nerve growth factor expression in the compound 48/80-treated HaCaT cells, an *in vitro* model for skin inflammation. Also, the anti-inflammatory activity of EPS was also confirmed in the animal model of contact inflammation of oxazolone-induced contact dermatitis of mice *in vivo*. With these backgrounds, we studied whether UVB-irradiation induced barrier dysfunction could be protected by EPS treatment via its antioxidant activity in NHEKs. As control experiments, NHEKs were also treated N-acetyl cysteine (NAC), a well-known antioxidant, to prove the efficacy of EPS in all of following experiments.

◯ EPS
- EPS stock solution was measured to contain trans-resveratrol at 176.75 ± 3.63 μg/mL. (Positive antioxidant control: NAC).

◯ Cell viability
- MTT assay of EPS in normal human epidermal keratinocytes (NHEKs)

◯ Measurement of intracellular ROS levels
- UVB-induced intracellular ROS was detected with a confocal microscopy using a DCF-DA.

◯ ROS-scavenging activity in NHEKs
- Tested biomarkers for inflammation cytokines : Interleukin-6 (IL-6), IL-8
- Tested biomarkers for MAPK pathway: pERK
- Tested biomarkers for barrier dysfunction: Keratin 1, Filaggrin (FIL), Involucrin

◯ ROS-mediated inflammation via phosphoAKT (pAKT)/ hypoxia-inducible factor-α (HIF-1α) pathway
- Tested inflammatory mediators for Akt-HIF-1α pathway: pAKT, HIF-1α

Results

1. Determination of optimal experimental conditions

In our search to find out the optimal experimental conditions, MTT assay demonstrated that < 1.6 mg/ml of EPS and < 4 mJ/cm^2 of UVB were safe without cytotoxicity to perform our experiments. Therefore, the following experiments were performed by treating NHEKs with 1 mg/ml EPS and 4 mJ/cm^2 of UVB (Figure 1).

2. EPS suppressed ROS production in UVB-induced NHEKs

To unravel the cytoprotective mechanism of EPS and NAC, we tested whether they had a potential to inhibit the UVB-induced ROS production in NHEKs. In DCF-DA staining, UVB produced DCF-DA-positive ROS from NHEKs, which was blocked by EPS and NAC treatment, respectively (Figure 2).

3. EPS downregulates expression levels of inflammatory biomarkers in UVB-irradiated NHEKs

UVB-induced upregulation of biomarkers for inflammation cytokines, such as IL-6, IL-8, COX-2 and ERK1/2, could be suppressed by treatments with EPS and NAC, indicating that ROS plays a crucial role to produce inflammatory cytokines in NHEKs (Figure 3A-C). Keratin 1, involucrin and filaggrin were also down-regulated by UVB irradiation, which were reversed by EPS and NAC treatments (Figure 3D).

4. EPS suppressed inflammatory mediators of pAKT and HIF-1α in UVB-irradiated NHEKs

In RT-PCR, UVB-irradiation induced the up-regulation of pAKT and HIF-1α in NHEKs, which were suppressed by treatments with EPS and NAC (Figure 4). The results suggest us that ROS plays a crucial role in inflammatory process via pAKT/HIF-1α pathway in keratinocytes .

5. EPS suppressed the NF-κB-mediated inflammation in UVB-irradiated NHEKs

From our *in vitro* results to demonstrate the anti-inflammatory activities of EPS, we examined the regulatory effects of EPS by assessing IκBα expression levels in NHEKs. In real-time PCR and Western blot analyses, UVB induced the downregulation of IκBα, an inhibitor of NF-κB, in NHEKs, which were reversed by EPS or NAC treatments. The results indicate that EPS and NAC have an anti-inflammatory activity by inhibiting NF-κB transcription in UVB-irradiated NHEKs (Figure 5A, B).

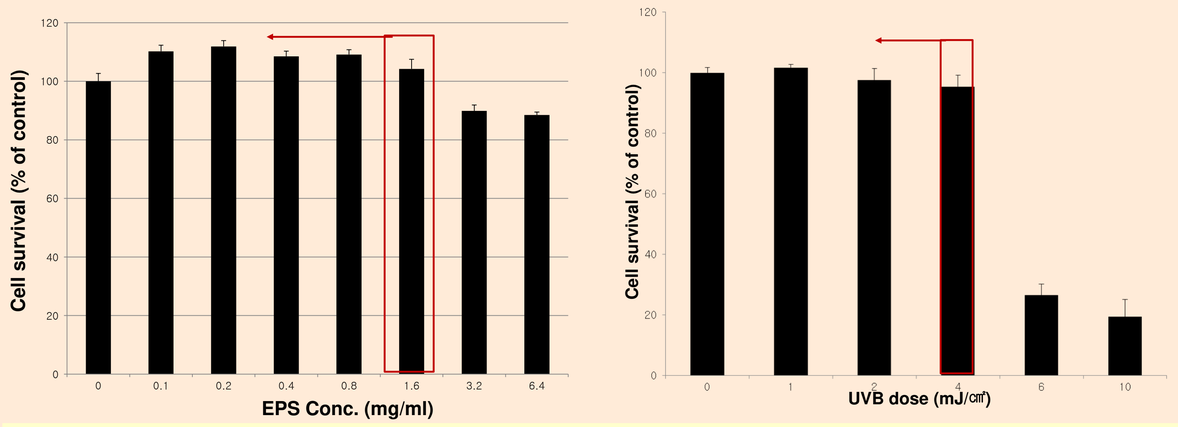

Figure 1. Set-up of non-cytotoxic conditions of EPS, UVB-irradiated NHEKs .

MTT assay to test cytotoxicity was performed after NHEKs were treated with the EPS and UVB irradiation for 24 h to decide optimal concentration of EPS and UVB doses for our experiments.

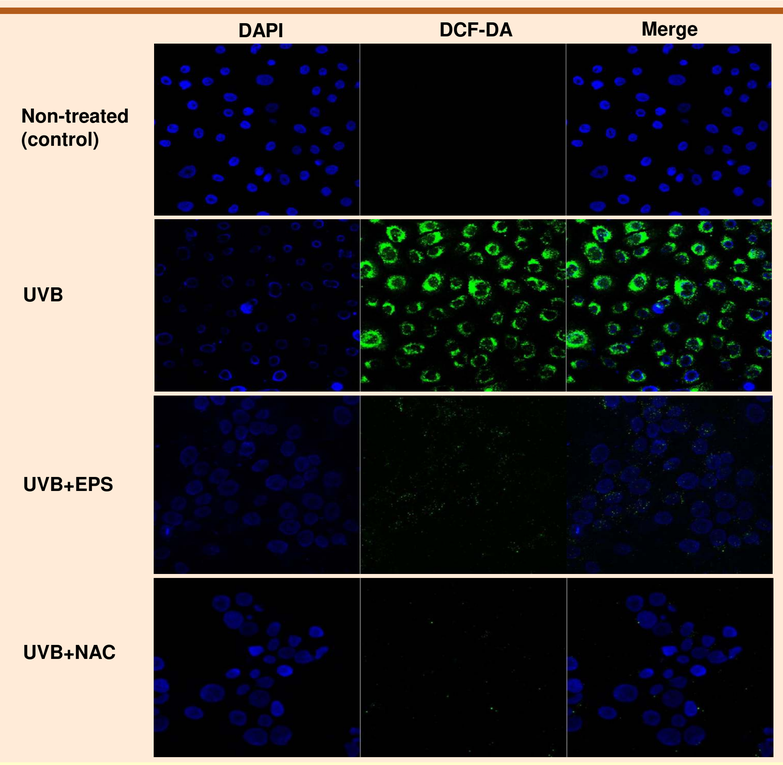

Figure 2. Effect of EPS on ROS production in UVB-irradiated NHEKs

After NHEKs were irradiated with UVB, cells were labeled with DCF-DA dye to detect intracellular ROS by a confocal microscope. Control NHEK without UVB irradiation, UVB-irradiated NHEKs, EPS-treated, and NAC-treated UVB-irradiated NHEK.

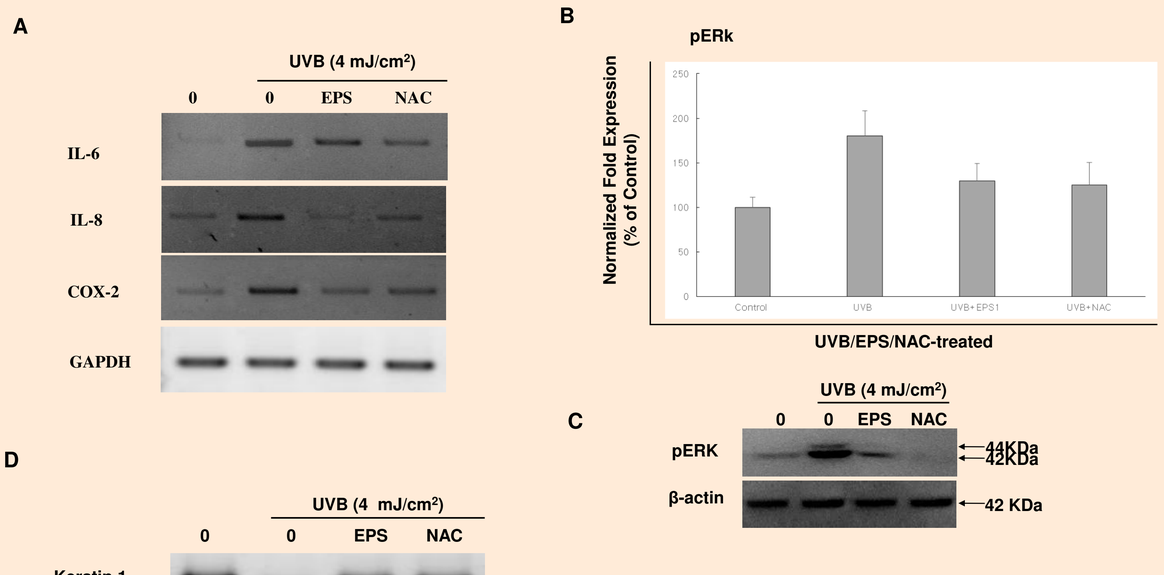

Figure 3. Modulation of inflammatory biomarkers by EPS in NHEKs

A. Expression levels of inflammatory biomarkers of IL-6, IL-8 and COX-2 were measured by RT-PCR experiments. B. Biomarkers for pERK cell signaling were checked with real-time PCR experiments. C. In Western blot analysis, UVB up-regulated pERK expression, which was reversed by EPS and NAC treatments. D. UVB-induced down-regulation of biomarkers for skin barrier (keratin 1, involucrin, filaggrin) were revsersed by EPS and NAC treatments.

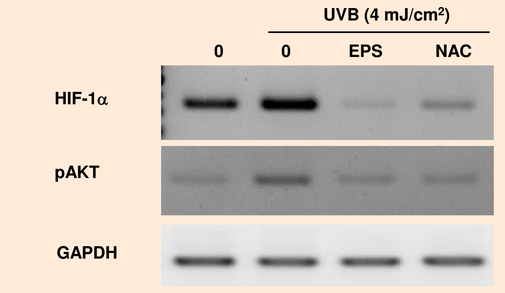

Figure 4. Modulation of pAKT and HIF–1α expression by EPS in NHEKs

In RT-PCR experiments, EPS and NAC showed activities to protect the UVB-induced upregulation of pAKT and HIF-1α in NHEKs.

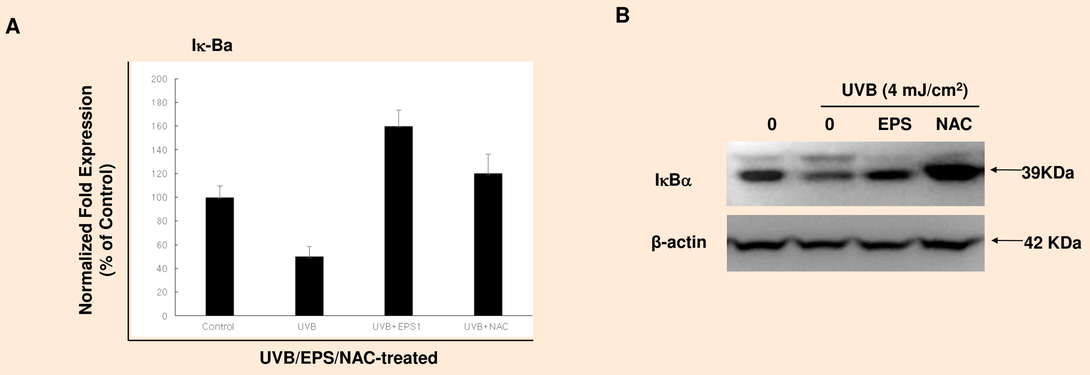

Figure 5. Modulation of IκBα expression, an inhibitor of NF-κB. by EPS in NHEKs

UVB-induced downregulation of IκBα was reversed by EPS and NAC treatments in NHEKs. A. In real-time PCR, the UVB-induced downregulation of IκBα was reversed by EPS and NAC treatments. B. In Western blot analysis, the UVB-induced downregulation of IκBα was also reversed by EPS and NAC treatments.

Summary

- ROS play an important role in the pathogenesis of inflammation biomarker in keratinocytes via IL-6, IL-8 as well as MAPK and Akt-HIF-1α pathways in keratinocytes.
- EPS is found to be a good candidate antioxidant to protect UVB-induced inflammation accompanying barrier dysfunction in keratinocytes. Further study is performing to develop a functional emollient including EPS as a new cosmeseutical product.

Acknowledgement

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