Edible bird’s nest extract downregulates epidermal apoptosis and helps reduce damage by ultraviolet radiation in skin of hairless mice

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(Received 30 April, 2021; Accepted 2 June, 2021)

The purpose of the present study was to examine whether daily intake of edible bird’s nest extract reduced ultraviolet-induced damage to skin. Twenty-one female HR-1/Hos mice were divided into control (C, n = 7), low-dose (2 mg/kg BW/day of edible bird’s nest extract) (L, n = 7), and high-dose (20 mg/kg BW/day of edible bird’s nest extract) (H, n = 7) groups. With their left back skin covered with aluminum sheet to prevent exposure, mice were radiated with either ultraviolet A (20 J/cm²) or ultraviolet B (40 mJ/cm²) in an alternate manner once daily for 10 weeks. They were gavaged either a solution of saline or edible bird’s nest extract every day. The moisture content of the ultraviolet-exposed right back skin was significantly higher in H than in C or L. Histochemical analysis showed that the number of apoptotic epidermal cells on the ultraviolet-exposed skin was significantly lower in L and H than in C. In H, the mRNA expression of superoxide dismutase 2 was significantly higher on ultraviolet-exposed skin than on unexposed skin. Our data suggested that edible bird’s nest extract enhanced superoxide dismutase 2 expression and downregulated apoptosis in their epidermis, which likely helped reduce skin damage.

Key Words: apoptosis, edible bird’s nest extract, skin damage, superoxide dismutase, ultraviolet

Edible bird’s nest (EBN) is a well-known Chinese dish. EBN contains regurgitated saliva of male swiftlets, and thus it is rich in mucinous glycoproteins such as chondroitin glycosaminoglycans and sialylglycoconjugates.1,2 Consecutive supplementation with EBN is believed to confer beneficial effects such as healthy skin and hence it is often used for medical purposes. Previous studies showed that EBN exhibited epidermal growth factor (EGF)-like activity.3,4 Furthermore, digested EBN has been reported to inhibit melanogenesis in vitro.5,6 We also reported that supplementation with EBN extract (EBNE) maintained dermal thickness of ovariectomized rats.7 However, to date, there is little information of the effect of EBN supplementation on molecular and cellular processes, which could help improve the health of the host’s skin.

Exposure of skin to ultraviolet (UVA and UVB) induces oxidative stress which can lead to serious damage of the epidermis. Both UVA (320–400 nm in wavelength) and UVB (290–320 nm) can penetrate the epidermis and induce DNA damage and apoptosis of epidermal cells.8–12 Repeated X-ray radiation also increased radicals derived from melanin in the hair and tail skin of mice.13 Exposure of urban particulate matter to epidermal keratinocytes induced oxidative stress and inflammation, and the supplementation of glycogen enzymatically synthesized from plant starch suppressed the inflammation by decreasing the accumulation of reactive oxygen species (ROS),14 which suggest the significance of alimentary intervention for the improvement of skin dysfunction. Past work reported that EBNE could induce production of ROS and improve cell viability in neuroblastoma cells11 and that it could downregulate the expression of apoptosis marker genes in rat brain.12 Superoxide dismutases SOD1 and SOD2, also known as Cu/Zn-SOD and Mn-SOD, respectively, are ubiquitous enzymes that efficiently catalyze dismutation of superoxide anions.13 In other words, superoxide anions generated by UV irradiation can be dismutated by these SODs to hydrogen peroxide, which is then detoxified by other antioxidant enzymes.14 Furthermore, a previous study showed that the activity of SOD2 increased in vitro after UVA radiation, in time- and dose-dependent manners.15 Based on this previous evidence, we theorized that supplementation with EBNE could also exhibit the additive effect of SOD activity, which in turn could help reduce oxidative stress and apoptosis of epidermal cells caused by UV radiation and hence, skin damage.

In the present study, we examined the antioxidant effect of EBNE orally supplemented on a daily basis, on the UV-damaged skin of hairless mice. Furthermore, to examine whether EBNE had an additive effect to UV radiation, we evaluated the expression levels of SOD in UV-exposed and unexposed epidermis obtained from the EBNE-treated hairless mice.

Materials and Methods

Animal experiments. All experimental procedures were approved by the Ethics Committee for Experimental Animal Use and Care of Kyoto Institute of Nutrition & Pathology (approval number: 10026CM). The experiment procedure for the UV-radiated mouse model was similar to that of a previous study,16 with some modifications. Briefly, 21 5-week-old female HR-1/Hos mice were purchased from a commercial breeder (Japan SLC, Shizuoka, Japan). Mice were randomly and equally divided (n = 7) into control (C), low-dose (2 mg/kg BW/day of EBNE) (L), and high-dose (20 mg/kg BW/day of EBNE) (H) groups. Enzymatically-digested EBNE (Collocalia; Combi Corporation, Tokyo, Japan) was dissolved in a saline solution, and approximately 0.1 ml of this solution was gavaged to mice with a commercial disposable feeding needle, on a daily basis (Fuchigami, Kyoto, Japan).

Mouse groups were housed in an environment-controlled (23–25°C; 40–60% humidity; 12:12 h light–dark cycle) room and kept in separate plastic cages. Throughout the study, mice were given semi-purified chow (AIN-93G, Research Diets Inc., NJ) as the basal diet and tap water ad libitum. Supplementation with

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doi: 10.3164/jcbn.21-54
Supplementation with EBNE

**Fig. 1.** Schematic presentation of the present experiment with edible bird’s nest extract (EBNE) supplementation and ultraviolet ray (UV) radiation. Oral supplementation with EBNE was conducted daily (arrows). After one week, radiation with either UVA (white arrowhead) or UVB (black arrowhead) was conducted in an alternate manner once daily for 10 weeks. Oral supplementation with EBNE was continued during the UV radiation period.

| Gene name | Primers 5'-3' | GenBank accession number |
|-----------|---------------|--------------------------|
| Superoxide dismutase 1 (Cu/Zn-SOD) (Sod1) | F ctctcaggagacattcactat<br>R caggaaattgtactgcga<br>P cctgcaaatgggtttcagagaacaa | NM_011434.1 |
| Superoxide dismutase 2 (Mn-SOD) (Sod2) | F gaacctcagtaagctggaaga<br>R aacgccacgaggagaagt<br>P tgaacactctcttggcagagcctc | NM_013671.3 |
| Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) | F gggttcttcaccacattga<br>R cagaaggggaggagatgat<br>P aagggccgggccactggaa | NM_008084.2 |

Primers and probes were designed and synthesized by Bioreseach Technologies Japan (Tokyo, Japan).

EBNE started as soon as the mouse groups were divided, and a week after, mice were subjected to UV radiation for 10 weeks. Mice were exposed to either UVA (20 J/cm²) for 50 min or UVB (40 mJ/cm²) for 30 s in an alternate manner once daily, with the skin of their left back covered with an aluminum sheet (1 cm × 3 cm) to block UV radiation. In a preliminary work, we radiated UVA and UVB for 10 weeks to hairless mice given EBNE, but no skin damage was observed (unpublished data). Therefore, in the present study, to cause severe oxidative stress in the skin, mice were exposed to UVB for 30 min, not 30 s, on one day in week 8.

Prior to dissection of mice, the moisture content of skin in the right back (UV-exposed side) was measured using a commercial moisture meter (Moisture-checker MY-808S; Scaral, Tokyo, Japan). For dissection, all mice were first deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (Somnopentyl; Kyoritsu, Tokyo, Japan). Samples were then collected from skin in both the right and left back sides of each mouse. Portions of the skin samples were immediately soaked in RNA-later solution (Thermo Fisher Scientific, MA) and kept at 4°C overnight, after which they were stored at −80°C until the mRNA expression analysis was conducted. The remains of the skin samples were fixed with 10% neutralized formaldehyde solution for histological staining. The procedure of the animal experiments is schematically shown in Fig. 1.

**Histological staining.** The fixed skin samples were embedded into paraffin wax and cut into 3-μm thick sections. To detect DNA fragmentation, the skin sections were then stained with terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL; *In situ* Apoptosis Detection Kit, Takara Bio, Shiga, Japan). One-cm digital images of the TUNEL-stained skin sections were captured using a light microscope equipped with a digital camera (DP25, Olympus). Afterwards, the number of TUNEL-positive nuclei in the epidermis of mice were counted.

**Gene expression analysis.** The methods for the RNA extraction and cDNA synthesis were the same as those previously described. Real-time polymerase chain reaction (PCR) was conducted using a Rotor-Gene 6200 (Qiagen, Tokyo, Japan). Primers and TaqMan probes used in this study are listed in Table 1. Optimal primers and probes were obtained from Bioresearch Technologies Japan (Tokyo, Japan). The methods employed for PCR analysis were the same as those previously described.

**Statistical analysis.** To estimate significant differences in TUNEL-positive cell numbers and the relative gene expression between groups, two-way analysis of variance (ANOVA; factors UV radiation and EBNE supplementation) and Tukey-Kramer post hoc comparisons were used. One-way ANOVA followed by Tukey-Kramer post hoc comparisons were used to evaluate significant differences in the moisture content between groups. Grubbs’s test was used to eliminate outliers. Data were considered statistically significant if \( p<0.05 \). Values are shown as the means ± SE.

**Results**

**Moisture of the skin after UV radiation.** The skin moisture contents in the skin of different mouse groups at week 10 are shown as percentages in Fig. 2. The skin moisture content was found to be significantly higher in H than in C (\( p<0.01 \)) or L (\( p<0.05 \)).

**DNA damage in epidermis.** Figure 3 shows the number of TUNEL-positive cells in the epidermis collected from left back skin (−UV) and right back skin (+UV). According to the results of the 2-way ANOVA, an interaction effect was observed. Therefore, we conducted multiple comparison tests to detect the differences between six groups (+UV_C, +UV_L, +UV_H, −UV_C, −UV_L, −UV_H). In C, the number of TUNEL-positive cells was found to be significantly (\( p<0.01 \)) higher in the epidermis of +UV skin compared with that of −UV skin. In addition, the...
number of TUNEL-positive cells was significantly lower in +UV skin of L ($p<0.001$) and H ($p<0.01$) than in that of C.

**Gene expressions of antioxidant enzymes.** Figure 4 shows the expression of Sod1 and Sod2 in +UV and −UV skins. The gene expression of Sod1 did not significantly differ after UV radiation and EBNE supplementation (Fig. 4A). Nonetheless, the 2-way ANOVA of Sod2 showed an interaction effect. Therefore, we conducted multiple comparison tests to detect differences between the aforementioned six groups again. The expression of Sod2 was significantly ($p<0.05$) higher in +UV skin of H than it was in −UV skin of the same mice (Fig. 4B).

**Discussion**

The results from the present study showed that although exposure to UV for 10 weeks stimulated apoptosis in the epidermis of mice, daily oral supplementation with EBNE helped reduce skin damage induced by this UV radiation.

Similar to the results from the present study, other recent studies conducted elsewhere also showed the protective effects of EBN against oxidative stress. For example, Murugan et al. showed that EBN normalized ROS production and oxidative stress in mouse models of hyperglycemic aorta and endothelial cells. This effect was also observed in hippocampal samples of a rat model of lipopolysaccharide-induced neuroinflammation. Moreover, EBN supplementation was reported to enhance antioxidant activity in blood and mRNA expression of antioxidant genes in the liver of obese mice fed a high-fat diet. The gene expression related to neurodegeneration and apoptosis in the hippocampus and frontal cortex of ovarioctomized rats also improved after EBN supplementation. EBN supplementation was also found to help reduce apoptosis and ROS production in the human neuroblastoma cells of a Parkinson’s disease model. We believe that, however, the present study is the first work to show positive effects of EBN supplementation on apoptosis reduction in the skin caused by oxidative stress.

In the present study, an interaction between EBNE and UV exposure was observed in the expression of Sod2; however, it was not the case with Sod1 expression (Fig. 4). While the reason for this discrepancy in the expression of SODs remains unclear, one possible explanation would be that the radiation dosage of UVA was much higher than that of UVB. The expression of Sod1 is reportedly elevated by UVB radiation. In contrast, UVA radiation increases SOD2 activity in vitro, which seems to imply that UVA radiation stimulate the gene transcription of Sod2 but not Sod1. Thus, it could be possible that in the present work, UVA and UVB differentially regulated the gene transcription of cytoplasmic Sod1 and mitochondrial Sod2. We suggest that different irradiation patterns be studied to elucidate the underlying molecular mechanisms in UV-damaged skin following supplementation with EBN.

The moisture content after 10 weeks of UV radiation was higher in H than in the other mouse groups. Furthermore, our results showed that EBNE reduced epidermal apoptosis. These results suggest that daily supplementation with EBNE helped...
maintain the protective epidermal barrier function. A reduced capacity of skin to retain moisture can lead to dry skin, which is associated with impairment of the epidermal barrier function.23,24

Although the present work did not examine the mechanisms by which oral supplementation with EBNE would exert positive effects on epidermal cells, in vitro supplementation with EBNE to cultured cells was shown elsewhere to promote cell viability and to exhibit EGF-like activity.3,11,23 Therefore, it is possible that certain components in EBNE act directly on EGF receptors found on the surface of epidermal cells and help increase cell viability. Further investigation is needed to identify the key components in EBNE that confer anti-apoptotic effects to epidermis.

In the present work, the dose of EBNE for H was 20 mg/kg BW/day. This dose would correspond to about 1–2 g/day for humans, which would be a reasonable daily oral intake. Therefore, our results highlighted the significance of EBNE supplementation for a possible preventive and/or therapeutic use to treat damaged skin of both animals and human.

Author Contributions

YM-I, TI and TT designed the experiments. YM-I and TI supplied the materials. TT conducted the experiments. SM carried out the statistical analysis and wrote the draft of the manuscript. TT was responsible for the overall direction of the project and for editing the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors thank Drs. Noriko Matsukawa and Keizo Nakayama, and Mr. Motohiro Nishikawa of Kyoto Institute of Nutrition & Pathology for their excellent technical assistance. We also thank Bioscience Proofreaders (https://bioscience-proofreaders.com) for editing the English language of the manuscript. This research received no external funding.

Conflict of Interest

YM-I and TI are employed by Combi Corporation. SM and TT are employed by Kyoto Institute of Nutrition & Pathology, which has received research funding from Combi Corporation.

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