Orally Administered Polysaccharide Derived from Blackcurrants (Ribes nigrum L.) Improves Skin Hydration in Ultraviolet-Irradiated Hairless Mice

Hiroshi ASHIGAI1, Yuta KOMANO1, Guanying WANG1, Yasuji KAWACHI1, Kazuko SUNAGA2, Reiko YAMAMOTO3, Ryoji TAKATA1 and Takaaki YANAI3

1 Research Laboratories for Health Science & Food Technologies, Kirin Co., Ltd., 1–17–1 Namamugi, Tsurumi-ku, Yokohama 230–8628, Japan
2 Marketing Department, Mercian Corporation, 4–10–2 Nakano, Nakano-ku, Tokyo 164–0001, Japan
3 Research Laboratories for Wine Technologies, Kirin Co., Ltd., 4–9–1 Johnan, Fujisawa 251–0057, Japan

(Received September 1, 2017)

Summary Blackcurrants (Ribes nigrum L.) have various benefits for human health. In particular, a polysaccharide derived from blackcurrant was found to be an immunostimulating food ingredient in a mouse model. We named a polysaccharide derived from blackcurrant cassis polysaccharide (CAPS). In a previous clinical study, we reported that CAPS affects skin dehydration, demonstrating its effectiveness against skin inflammation was related to atopic dermatitis; skin inflammation caused skin dehydration. However, there are no studies regarding CAPS effectiveness against skin dehydration. The current study aimed to investigate CAPS effectiveness against skin dehydration. We further demonstrate the effect of oral administration of CAPS on skin dehydration caused by ultraviolet (UV) irradiation-induced inflammation in mice. We found that CAPS administration suppresses skin dehydration caused by UV irradiation. We also found that CAPS decreases interleukin-6 and matrix metalloproteinase transcription levels in the mouse skin. These results show that CAPS improves skin hydration in UV-irradiated mice.

Key Words blackcurrant, polysaccharide, ultraviolet, skin hydration

Materials and Methods

Blackcurrants were purchased from the Central Chemical Company (Tokyo, Japan). Crude blackcurrant juice was obtained, and the following glucanase enzymes were added: 0.01% Sclase S® (Mitsubishi-Kagaku Foods Corp., Tokyo, Japan) and 0.01% Viscozyme® (Novozymes Japan Ltd., Chiba, Japan). After incubation at 48˚C for 5 h, the enzymes were inactivated by raising the temperature to 80˚C for 10 min. Subsequently, the supernatant was recovered after centrifugation (8,000 rpm for 10 min). Ethanol precipitation was performed with a double volume of blackcurrant juice (1 L). The precipitate was recovered after centrifugation (8,000 rpm for 10 min), and then dissolved in a moderate volume (100 mL) of phosphate-buffered saline (PBS). Ethanol

Ultraviolet (UV) radiation can cause skin inflammation (1). It also induces reactive oxygen species (ROS) (2). There are three types of solar UV according to wavelength: UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (100–290 nm). In particular, UV-B induces DNA damage in the epidermis, which results in the formation of pyrimidine and purine photoproducts, such as pyrimidine dimers (2). These photoproducts can cause DNA strand breakage and generate HO• radicals, which also disrupt the skin barrier function (2, 3). The skin barrier has an important role in retaining skin moisture, immunomodulation, and skin aging (4–6). However, in cases of skin damage, the epidermis loses its intracellular lipid and natural moisturizing factors (7). As a result, UV radiation can cause dehydration in the epidermis (8). Chronic inflammation caused by UV radiation can also lead to skin cancer (9, 10). Given this evidence, we believe that protection from UV radiation is very important for skin hydration.

Inflammatory cytokines such as interleukin-6 (IL-6) induce matrix metalloproteinases (MMPs) (11); MMP-13 is one of the representative collagenases of the mouse skin (12), and collagen is one of the important factors for skin moisture (13). Thus, the levels of inflammatory cytokines and collagenase indicate the severity of skin inflammation.

Blackcurrant (Ribes nigrum L.) is a well-known healthy fruit that is used to prepare various foods and beverages, such as jams and juices. In previous studies, we revealed that a polysaccharide-rich substance derived from blackcurrants reduces skin inflammation linked to atopic dermatitis, and that it has an anti-tumor effect in mice (14–16). We designated this substance cassis polysaccharide (CAPS). It consists mainly of galactose and arabinose (14, 15).

Several polysaccharides used as food ingredients are known to have effects on the skin damage induced by UV (17–20). In this study, we investigated whether CAPS can improve skin hydration after UV irradiation in mice.
precipitation was repeated three times before lyophilization of the precipitate using a freeze dryer.

The molecular weight (MW) of CAPS was assessed by gel-filtration chromatography using a high-performance liquid chromatograph (HPLC; Shimadzu Corp., Kyoto, Japan) equipped with a Shodex OHpak® SB-805 HQ column (Showa Denko K.K., Tokyo, Japan) equilibrated with PBS at a flow rate of 1 mL/min. The detection analysis was performed using a RID-20A refractive index detector (Shimadzu Corp.). A calibration curve was plotted with the results obtained using standard solutions of dextran (T2000 [MW: 2,000,000], T500 [MW: 473,000], T70 [MW: 67,200], T40 [MW: 43,000], and T10 [MW: 10,000]) along with sucrose (MW: 342) and glucose (MW: 180). These markers were purchased from Pharmacosmos A/S (Holbaek, Denmark).

Forty specific-pathogen-free female Hol:HR-1 hairless mice (aged 5 wk old) were purchased from Japan SLC, Inc. (Yokohama, Japan). The mice were treated in accordance with the ethics guidelines for animal care, handling, and euthanasia of the study sponsor, Kirin Co., Ltd. The Committee for Animal Experimentation at Kirin Company approved the experiments (approval number: Y010-00169).

The mice were exposed to a 12:12-h light:dark cycle, with the light period beginning at 6:00 a.m. The temperature of the room was maintained at 24±2.0°C and the humidity was maintained at 55±10%. Purified rodent food (AIN-93G; CLEA Japan, Inc., Tokyo, Japan) and water were provided. After acclimation for 7 d, the mice were divided into 4 groups (10 mice per group) such that there were no significant differences in body weight or hydration of the stratum corneum among the groups. Two of the groups were control groups, and the mice in these groups were fed a diet consisting only of AIN-93G. The mice in the other two groups were fed a diet of AIN-93G containing either 0.2% or 1% CAPS. Two of the groups were control groups, and the mice in these groups were fed a diet consisting only of AIN-93G. The mice in the other two groups were fed a diet of AIN-93G containing either 0.2% or 1% CAPS powder. After 2 wk, the mice in one of the control groups and both the CAPS groups were irradiated with UV light (90 mJ/cm²) using the UV irradiation system SUPERCURE®-204S (San-È Electric Co., Ltd., Osaka, Japan). After UV irradiation, the diets were continued for an additional 4 d.

The stratum corneum hydration of the skin in the lumbar region was measured using a Corneometer® CM 825 (Courage + Khazaka Electronic GmbH, Köln, Germany) and the transepidermal water loss (TEWL) was measured using a Tewameter® TM 300 (Courage & Khazaka Electronic GmbH) (21, 22).

The mice were then euthanized, and skin samples were collected. Skin samples were frozen in liquid nitrogen and crushed into powder using Multi-beads shocker® MB2000 (Yasui Kikai, Osaka, Japan) (23).

To analyze the expression levels of the two target genes, IL-6 and MMP-13, real-time polymerase chain reaction (RT-PCR) was performed. The total RNA from epidermal cells was purified using a QIAshredder (Qiagen K.K., Tokyo, Japan) and an RNeasy® Mini Kit (Qiagen K.K.), according to the manufacturer’s instructions; cDNA was synthesized using a PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara Bio, Inc., Shiga, Japan). The expression levels of the target genes were analyzed using SYBR® Premix Ex Taq™ II (Takara Bio, Inc.), according to the manufacturer’s instructions. This involved adding 250 ng of each cDNA mixture to 20 μL of the reaction mixture. The primers used were as follows: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers, TCTGCTGATGCCCCTAGTTCG and TGGTGGCCATGATGCTAGGA; IL-6 primers, CTCTGGGAAATCTGTTGAAAT and CCAGTTTGGTAGCATCCATC; and MMP-13 primers, CCTGGAATTG-GCAACAAAGT and CCCACCCTATGACATGAAA. The reactions were performed using a LightCycler® 480 System (Roche Diagnostics GmbH, Mannheim, Germany).

The RNA expression levels for each group were quantified by comparisons with a standard curve plotted using the results obtained with cDNA templates (along with the relevant primers). Each target gene value was divided by the GAPDH gene value, and these relative gene expression levels were normalized to the value of the UV-untreated control group.

For each of the four groups, we calculated the mean (and standard error of the mean) stratum corneum hydration, TEWL, and relative gene expression levels of IL-6 and MMP-13. We performed Dunnett’s test to analyze between-group differences (compared to the UV-treated control group) using Ekuseru-Toukei® 2010 statistical software (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

We obtained 13.5 g freeze-dried powder from 3,454 g blackcurrant juice; the mean MW was 27,643. This result supports the earlier result that indicates that the freeze-dried powder mainly contains fiber (16).

With the Prosky method, the dietary fiber content was found to be 828 mg/g freeze-dried powder. This indicates that the freeze-dried powder mainly contained dietary fiber. We defined this component of the blackcurrant juice as CAPS.

There were no differences in food intake or body weight between groups (data not shown).

The stratum corneum hydration was significantly
Blackcurrant-Derived Polysaccharide Improves Skin Hydration after UV Irradiation

Fig. 2. Effects of the administration of a polysaccharide derived from blackcurrant (cassis polysaccharide [CAPS]) on transepidermal water loss (TEWL) after ultraviolet (UV) irradiation. The data are expressed as the mean±standard deviation (SD) (n=10). The differences between control (UV treated) and each test group were statistically significant by Dunnett’s test (p<0.05).

higher in both CAPS groups (in a concentration-dependent manner) than in the UV-treated control group (Fig. 1). In contrast, the TEWL was significantly lower in both CAPS groups than in the UV-treated control group (Fig. 2).

The results of RT-PCR showed that IL-6 and MMP-13 gene expression levels of both CAPS groups were lower than in the UV-treated control group (Fig. 3).

Discussion

It is known that UV radiation can induce inflammation in the epidermis (1), which in turn induces inflammatory cytokines, such as IL-6 (2). The stratum corneum hydration and TEWL are representative parameters of skin barrier function (24). In this study, UV radiation decreased stratum corneum hydration (Fig. 1) and increased TEWL (Fig. 2). Based on these results, we concluded that UV radiation disrupted skin barrier function.

The CAPS administration groups (0.2% CAPS, 1.0% CAPS) demonstrated suppressed stratum corneum dehydration and TEWL compared with the UV-treated control group (Fig. 1, Fig. 2). Our results indicate that administering CAPS improves skin hydration caused by UV irradiation.

HO• radicals are induced by UV radiation (2). They disrupt the antioxidative system in the skin (3), and can cause immune suppression, cancer, and skin aging (25). Catalase is a representative parameter of oxidation in epidermal tissue that can suppress active oxygen activity (26). In a previous study, we revealed that CAPS has an effect against the antioxidation induced by UV radiation in the human skin (27). In particular, CAPS suppresses catalase activity in the human skin (28). We assume that CAPS improves skin barrier function by suppressing skin oxidation.

It is known that UV radiation induces skin inflammation (28–30). Our results showed that IL-6 transcription in the skin was suppressed by CAPS administration. We assume that CAPS suppressed skin inflammation. In a previous study, we reported that CAPS had an anti-inflammatory effect in NC/Nga mice (10). Moreover, there are several other studies regarding polysaccharides, including galactose, that have been shown to reduce skin inflammation caused by UV irradiation (17–20). Our results are in line with these studies.

Skin inflammation causes extracellular matrix damage and MMP activation. Administration of CAPS suppresses MMP-13 transcription (Fig. 3). A fibroblast collagenase, MMP-13, is the key enzyme responsible for breaking down dermal components (31, 32). Once the MMP-13 activation initiates degradation of fibrillar type I and III collagen, further processing is performed by MMP-3 and MMP-9 (23). Therefore, MMP-13 plays an important role in the initiation of UVB-induced wrinkle formation and skin hydration. We assume that CAPS exerts an effect on skin wrinkle formation and anti-inflammatory effect via the nuclear factor kappa-light-chain-enhancer of activated B cells or ROS pathways.

In a previous study, we reported that CAPS administration activates IL-10 production in mice (14), which suppresses inflammation (33). Thus, we assume that CAPS may exert an effect on inflammation through IL-10 activation.

Regarding the limitations of this study, we did not explore the detailed mechanism of action underlying the demonstrated effects of orally administered CAPS. In future studies, we intend to elucidate the precise mechanism.

In conclusion, orally administered polysaccharide derived from blackcurrants (R. nigrum L.) improves skin hydration in UV-irradiated hairless mice.

REFERENCES


