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Oral administration of hyaluronan prevents skin dryness and epidermal thickening in ultraviolet irradiated hairless mice

Chinatsu Kawada [a], Mamoru Kimura [a,b], Yasunobu Masuda [a], Yoshihiro Nomura [b,*]

[a] R&D Division, Kewpie Corporation, Tokyo, Japan
[b] Faculty of Agriculture, Scleroprotein and Leather Research Institute, Tokyo University of Agriculture and Technology, Tokyo, Japan

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ABSTRACT

Hyaluronan is a component of the extracellular matrix that plays a role in water retention in tissues. In this study, we orally administered hyaluronans of varying molecular weights (300 k and less than 10 k) repeatedly to hairless mice exposed to ultraviolet (UV) irradiation and examined their effects on the skin of these mice. UV irradiation induces a marked increase in the epidermal thickness of the dorsal skin and a marked decrease in the skin moisture content; however, orally administered hyaluronan, particularly that with a molecular weight of less than 10 k, markedly reversed the increase and decrease in the epidermal thickness and skin moisture content, respectively. Furthermore, on analyzing the mice skin, orally administered hyaluronan with a molecular weight of less than 10 k increased the levels of the HAS2 gene expression in the skin. Based on these findings, it is assumed that orally administered hyaluronans, with molecular weight of 300 k and less than 10 k, reversed UV irradiation-induced skin disturbance. In particular, it was considered that the increase in the skin moisture content by orally administered hyaluronan, with a molecular weight of less than 10 k, was related to the effect on skin cells.

1. Introduction

Hyaluronan (HA) is a linear glycosaminoglycan that is a major component of the extracellular matrix, which is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine that are linked via alternating β-1, 4 and β-1, 3 glycosidic bonds [1]. In all vertebrates, more than 50% of the total body HA is present in the skin [2,3]. HA is responsible for regulating the water balance in the skin and maintaining the cell structure in the dermis [4], and it is involved in keratinocyte proliferation and differentiation in the epidermis [5–7].

Skin aging is divided into age-related intrinsic aging and ultraviolet (UV) irradiation-induced photoaging [8]. Photoaged skin is characterized by wrinkles, dryness, roughness, pigmented spots, histological changes, and decreased skin barrier function [9]. These symptoms are caused by decreased collagen [10], HA decomposition [11], increased matrix metalloprotease [12], reactive oxygen [13], and elastin accumulation [14] in the skin. Ingested HA increases the skin moisture contents in subjects with dry skin [15,16]. The orally administrated HA is absorbed into the body, and the orally administrated HA is then detected in the skin [17–19]. The orally administrated HA is reportedly absorbed intact [18] as well as in the form of its decomposed metabolites by intestinal bacteria [20]. In this study, we examined the preventive effects of orally administrated HA on skin photoaging.

2. Materials and Methods

2.1. Materials

Two types of HA that were produced by microbial fermentation at the Kewpie Corporation (Tokyo, Japan) were used. The molecular weights (MWs) of these HA were 300 k (Hyabest®(S) LF-P) and less than 10 k, which were determined by the analysis of limiting viscosity. All the other reagents used were special grade items that were produced by Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Animal Experiments

This study was approved by the Ethics Committee of the Tokyo University of Agriculture and Technology in accordance with the guidelines of the Swiss National Institute of Health (no. 23–29).

Six-week-old HR-1 hairless male mice were obtained from Sankyo Labo Service Corporation, Inc (Tokyo, Japan). The animals were kept on a 12-h light/dark cycle at 24 °C ± 2 °C with 55% ± 5% humidity, and they had free access to the Labo MR Stock (Nosan Corporation, Kanagawa, Japan) and sterile distilled water.

* Corresponding author at: Scleroprotein and Leather Research Institute, Tokyo University of Agriculture and Technology, 183-8509 Saiwai-cho, Fuchu-shi, Tokyo 183-0057, Japan.
E-mail address: ny318@cc.tuat.ac.jp (Y. Nomura).

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2.3. Experimental Design and Oral Administration

The animals were allowed to acclimatize for a week before the start of the experiment. Twenty-three mice were allocated to the following four groups: the non-UV irradiated group [UV(−) control group, n = 6], which was a negative control group, the UV-irradiated group [UV(+) control group, n = 6], the UV-irradiated and HA-treated (MW, 300 k) group [UV(+) HA300 k group, n = 5], and the UV-irradiated and HA-treated (MW, less than 10 k) group [UV(+) HA10 k group, n = 6]. These groups were adjusted such that they had the same average body weight and skin moisture content. The HA were dissolved in sterile distilled water, and the mice in the HA-treated groups were administered oral HA at a dose of 200 mg/kg body weight per day for six weeks with concurrent exposure to UV irradiation three times and skin moisture content measurements twice per week. After the experimental period, the mice were sacrificed by the collection of whole blood from their hearts under the effect of anesthesia (SEVOFRANE®; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan). Samples that were obtained from the dorsal skin were rapidly frozen in liquid nitrogen and stored at −80 °C. Skin biopsy samples that were removed using a biopsy punch with a diameter of 8 mm for the histological analysis were fixed in 10% buffered formalin.

2.4. Ultraviolet Irradiation

In accordance with the UV-irradiation method of Tanaka et al. [21], the mice were housed in a stainless steel cage (5 × 9 × 4 cm) and subjected to UV irradiation that was emitted from a UV-B lamp (GL20SE; Sankyo Denki Co., Ltd., Tokyo, Japan). The UV irradiation was performed for 1 min and 30 s each time in the first week. The exposure time was then increased to 2 min each time 3 times a week in the second week, 2 min and 30 s each time in the third week, 3 min each time in the fourth week, 3 min and 30 s each time in the fifth week, and the final duration of 3 min and 45 s each time in the sixth week, resulting in the total irradiation of 2.8 J/cm^2 in each mouse.

2.5. Histological Analysis

Dorsal skin was fixed in formalin, embedded in paraffin, and prepared for optical microscopy. Hematoxylin & Eosin (H&E) staining was conducted for tissue examinations and to measure the epidermal thickness. Three sites were randomly selected in the sections from each mouse, and the thickness of the epidermis was measured in 10 points per site under the microscope with Axio Vision software version 4.5 (Carl Zeiss Microscopy Co., Ltd., Tokyo, Japan). The mean of these three measurements in each mouse was used to calculate the mean for each experimental group.

2.6. Skin Moisture Content

The skin moisture content of the dorsal skin was measured with a Corneometer CM825 (Courage + Khazaka electronic GmbH, Colognen, Germany) after it was kept at 20 ± 2 °C and at 50 ± 5% humidity for 1 h prior to the HA administration and twice a week during the experimental period. The final value for the day was the average of 1 s × five measurements, and the final value for the week for each mouse was the average of the twice-a-week measurements.

2.7. Transepidermal Water Loss

Transepidermal water loss (TEWL) of the dorsal skin was measured with Tewameter TM300 (Courage + Khazaka electronic GmbH, Colognen, Germany), similar to the measurement of the skin moisture content, after it was maintained at 20 °C ± 2 °C and at 50% ± 5% humidity for 1 h on the day before the dissection at the end of test. TEWL was measured at 1 s intervals by vertically pushing the probe into the dorsal skin of the waist, under anesthesia (SEVOFRANE®; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan). When the standard deviation of five continuously measured values was under 0.1, the average of the last five measured values was used as the TEWL value for each mouse.

2.8. Quantitative Real-time Polymerase Chain Reaction

The skin samples were homogenized in TRIzol Reagent (Life Technologies Corporation, Grand Island, NY, USA), and homogenates were centrifuged at 10,000 rpm for 15 min at 4 °C. The total RNA that was obtained from those supernatants was used for cDNA production with a PrimeScript RT reagent Kit (Perfect Real Time; Takara Bio Inc., Shiga, Japan). Quantitative real-time polymerase chain reaction (RT-PCR) was performed with the intercalater method and SYBR Green I with the Thermal Cycler Dice Real Time System TP800 (Takara Bio Inc.). The measurement of each sample was done with doublet holes, and the fluorograms were examined after 40 cycles of PCR for hyaluronan Synthase 2 (HAS2) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The sequences of the primer pairs, 5′ and 3′, were as follows: HAS2, gtcatgtacacagccttcagagcac and ggcagggtcaagcatagtatctgag; and GAPDH, tgtgtccgtcgtggatctga and ttgctgttgaagtcgcaggag, respectively. The housekeeping gene GAPDH was used for internal normalization. The analysis of the quantitative RT-PCR data was conducted with the machine exclusive software (Thermal Cycler Dice Real Time System TP800 Software, Ver.1.02A).

2.9. Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). For the skin moisture content, TEWL, and epidermal thickness, which were markedly affected by UV irradiation, the UV(−) control group was considered as a reference, and Dunnett test was used as the standard with the UV(+) control group to compare the values between the groups. In contrast, for the HAS2 gene expression in the skin, which was not markedly affected by UV irradiation, Tukey–Kramer's test was used to compare the values between the groups. All statistical analyses were performed with the SPSS software package (IBM Corporation, Armonk, NY, USA). p values of less than 0.05 were considered statistically significant, and those less than 0.10 were considered as having a statistically significant tendency.

3. Results

3.1. Histopathological Analysis of the Dorsal Skin

Fig. 1 shows histopathological images of the UV-irradiated dorsal skin of hairless mice, and Fig. 2 shows the measurement results of epidermal thickness. In the skin of the UV(−) control group (Fig. 1(a)), the epidermis was thin with a thickness of two to three cell layers (19.14 ± 0.44 μm). A hyperplastic response with six to eight cell layers was evident in all the skin that had been exposed to UV irradiation (Fig. 1(b)–(d)). The UV(+) control group exhibited a significant increase in epidermal thickness compared with the UV(−) control group (Fig. 2, $p < 0.01$). However, the increase in epidermal thickness was suppressed in the UV(+) HA300 k group and the UV(+) HA10 k group, and the epidermal thickness decreased in the UV(+) HA300 k group by 18% and in the UV(+) HA10 k group by 26% as compared with the epidermal thickness of the UV(+) control group. In particular, the UV(+) HA10 k group exhibited a significant tendency for a decrease in the epidermal thickness as compared with the UV(+) control group ($p < 0.10$).