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Note

Effects of Collagen Peptide Ingestion on UV-B-Induced Skin Damage

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The effect of daily ingestion of collagen peptide on the skin damage induced by repeated UV-B irradiation was examined. Ingestion of collagen peptide (0.2 g/kg/d) suppressed UV-B-induced decreases in skin hydration, hyperplasia of the epidermis, and decreases in soluble type I collagen. These results suggest that collagen peptide is beneficial as a dietary supplement to suppress UV-B-induced skin damage and photoaging.

Key words: photoaging; type I collagen; skin hydration

Collagen is the most abundant protein in the vertebrate body, comprising about one-third of total protein. Collagen extracted with hot water from animal bone, hide, or fish scales is called gelatin, and its hydrolysate is often called collagen peptide (CP) when used as a supplement. Ingestion of gelatin or CP affects various functions of the body, including bone,[1)] the Achilles tendon,[2)] and skin[3)] and skin appendages.[4)] One of the outer insults that damage the skin is ultraviolet irradiation. Ultraviolet is divided into three categories according to wavelength: UV-A (400–315 nm), UV-B (315–280 nm) and UV-C (<280 nm). Repeated skin exposure to UV-B results in an aged skin phenotype (photoaging), including wrinkle formation. Although ingestion of CP has various beneficial effects on the body including the skin,[3)] it remains unknown whether UV-B-induced skin injury is affected by ingestion of CP. In this study, we administered CP prepared from fish scale to hairless mice repeatedly exposed to UV-B irradiation for 6 weeks, and UV-B-induced skin damage was examined.

All animal experiments were approved by the Ethics Committee of Tokyo University of Agriculture and Technology. Six-week-old male Hos:HR-1 hairless mice (SLC Japan, Tokyo) were housed in collective cages at $20 \pm 2\,^{\circ}C$ on a 12-h light/12-h dark cycle, with free access to water and Labo MR Stock diet (Nosan, Tokyo, Japan). After 5 d of acclimation, mice were divided into three groups (seven mice per group) such that the body weight and hydration of the stratum corneum did not differ significantly among groups. CP (FCP-A, Nippi, Tokyo; derived from scales of *Tilapia zillii*) was dissolved in distilled water at 0.025 g/ml and administered p.o. at 0.2 g/kg, body weight daily. The mice were housed in a stainless steel cage ($5 \times 9 \times 4$ cm) and subjected to UV-B irradiation ($0.3\,mW/cm^2$) emitted from a UV-B lamp (GL20SE; Sakyo Denki, Tokyo). UV-B was irradiated 3 times per week 1 min each time, in the first week. The exposure time was then increased to 2 min × 3 times per week in the 2nd week, 3 min × 3 times in the 3rd week, and 4 min × 2 times in the 4th week, and was finally maintained at 3 min × 7 times in the 5th and 6th weeks (total energy, 0.846 J/mouse).

Hydration of the stratum corneum of the lumbar skin was measured once a week with a Corneometer CM 825 (Courage+Khazaka Electronic, Köln, Germany) after being kept at $20 \pm 2\,^{\circ}C$ and $50 \pm 5\%$ humidity for 2 h. After the 6-week experimental period, the mice were sacrificed by cervical dislocation, and seven skin samples from each group were assembled and subjected to extraction of protein.[1)] The extracted type I collagen was visualized by western blot using rabbit antiserum raised against porcine skin-derived type I collagen[1)] as the first antibody, and horseradish peroxidase-labeled mouse monoclonal IgG anti-rabbit IgG was employed as the second antibody.[1)] Skin samples were also obtained after the 6-week experimental period and were prepared for histological examination. Four sites were randomly selected in sections from each mouse, and the thickness of the epidermis was measured under the microscope using Axio Vision software (version 4.5, Zeiss, München, Germany). The mean of four values for each mouse was used to calculate the mean and SD for each experimental group. Differences in the mean for each group were analyzed by the Tukey-Kramer method using Prism 4 software (MDF, Tokyo).

Throughout the experimental period, body weight did not differ significantly among the non-irradiated mice [UVB(−)], the UV-B-irradiated mice (UVB), and the UV-B-irradiated mice fed CP (UVB+collagen) (data not shown). After 3 weeks, hydration of the stratum corneum in the UVB group was significantly lower than that in the UVB(−) group. However, the hydration of the stratum corneum did not decrease significantly in the UV-B-irradiated mice fed CP (UVB+collagen) compared to UVB(−) (data not shown). After 5 weeks (Fig. 1) or 6 weeks (data not shown), skin hydration in the UVB+collagen group was significantly higher than in the UVB group. The results, in Fig. 1 suggest that ingestion of CP suppresses UV-B-induced change in the outermost region of the skin, the stratum corneum of the epidermis. Therefore, we examined the effects of CP ingestion on the thickness of the epidermis. The thick-

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Abbreviations: CP, collagen peptide; TBS, Tris-buffered saline; PB, phosphate buffer; Pro-Hyp, prolyl-hydroxyproline

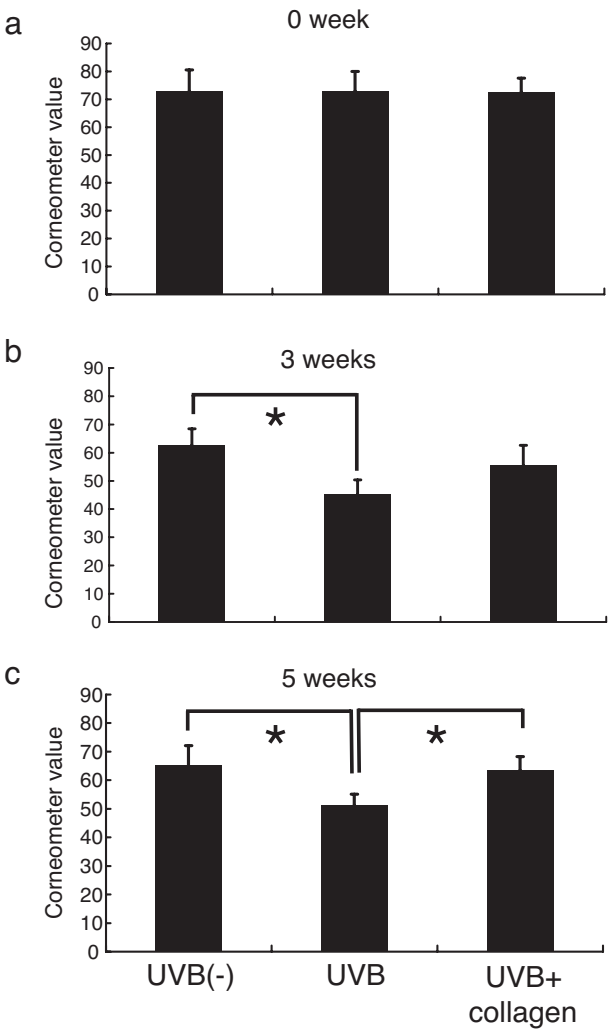

Fig. 1. Effects of UV-B Irradiation and CP Ingestion on Hydration of the Stratum Corneum.

The hydration of the stratum corneum in non-irradiated (UVB(−)) mice, UV-B-irradiated (UVB) mice, and UV-B-irradiated, and CP-administered (UVB+collagen) mice (7 mice per group) was measured with a Corneometer at 1-week intervals. Values before the experiment (a) and at 3 weeks (b) and 5 weeks (c) are shown. *Significant difference between the UVB(−) and UVB or between the UVB and UVB + collagen groups ($p < 0.05$).

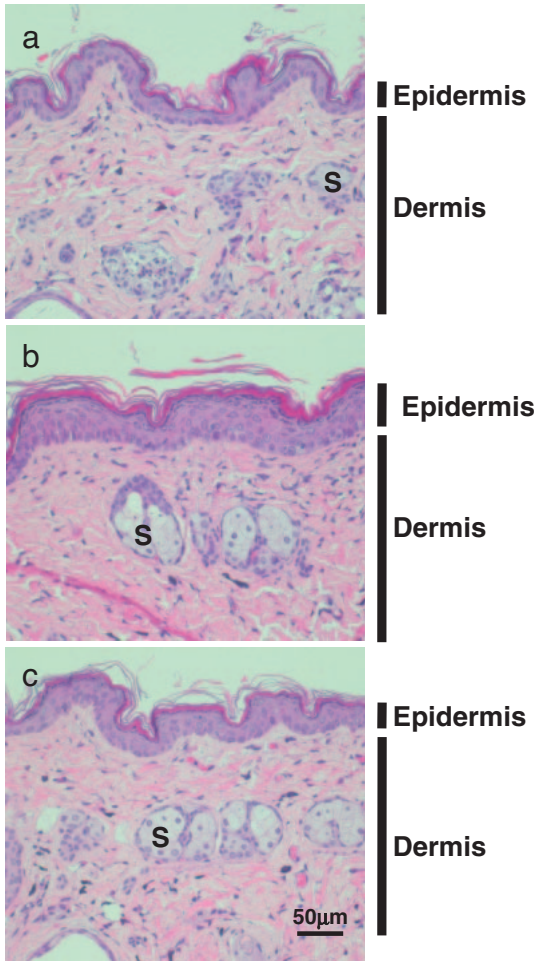

Fig. 2. Histology of the Skin.

Skin samples from each mouse were taken after the 6-week experimental period, and thin sections were stained with Hematoxylin and Eosin. a, the UVB(−) group; b, the UVB group; c, the UVB+collagen group. S, sebaceous gland. Bar, 50 μm.

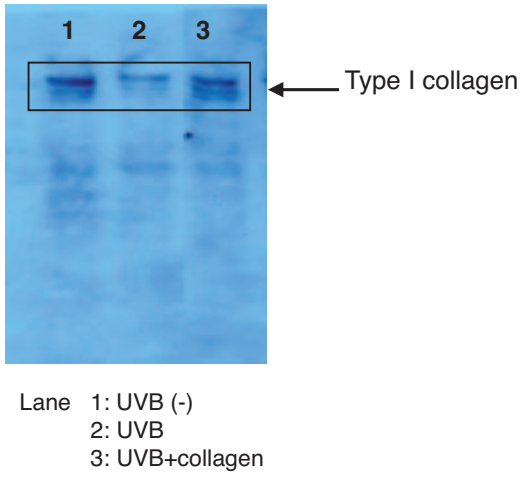

Fig. 3. Western Blot Analysis of Soluble Type I Collagen in the Skin.

Soluble type I collagen was detected using anti-porcine type I collagen antibody. Lane 1, the UVB(−) group; lane 2, the UVB group; lane 3, the UVB+collagen group.

ness of the epidermis in the UVB (Fig. 2b, 37.8 ± 9.5 μm) was significantly larger than that in the UVB(−) group (Fig. 2a, 27.1 ± 5.9 μm) at 6 weeks. In contrast, this increase in epidermal thickness was suppressed by CP ingestion (Fig. 2c, 31.1 ± 5.6 μm), and no significant difference was seen between the UVB(−) group and the UVB+collagen group. The effect of UV-B on the dermis was examined by Western blot analysis of soluble type I collagen.[5] The soluble type I collagen decreased markedly under repeated UV-B irradiation for 6 weeks (Fig. 3, the lanes 1, 2); the relative amount of type I collagen in the UVB group was 47, and the UVB(−) group had a relative value of 100. However, the decrease in soluble type I collagen was evidently suppressed by CP ingestion (relative amount, 117; Fig. 3, lane 3). These results suggest that the skin change in either the epidermis or the dermis is suppressed by daily ingestion of CP. Thus the present study indicates that ingestion of collagen peptide can suppress UVB-induced damage to the skin.

Chronic exposure to UV-B irradiation is known to damage skin structure and function, and induces photoaging, characterized by wrinkles, laxity, roughness, and irregular pigmentation. UV-B irradiation induces the

production of reactive oxygen species (ROS) that damage the anti-oxidative defense mechanisms of the skin, which results in immune suppression, cancer formation, and premature skin aging through the oxidation of cellular and non-cellular components. The mechanism of photoaging has been reviewed by Yaar and Gilchrest.[6] ROS activate cell surface receptors such as epidermal growth factor receptor (EGFR) and lead to intracellular signaling. Expression of nuclear factor AP-1 is induced by activated kinases, UV-B-induced cysteine-rich 61 protein (CYR61), or ROS themselves. Increased AP-1 transcription and its activity interfere with the synthesis of collagen and up-regulate the matrix degrading enzymes MMP-1 and MMP-3. It also blocks the effect of transforming growth factor-β (TGF-β), suppressing collagen gene expression and activating keratinocyte proliferation. Elevated epidermal proliferation (hyperplasia) and decreased collagen production are partly induced by UV-B-induced SMAD7 expression too. On the other hand, UV-B irradiation activates another nuclear factor, NF-κB, which results in the expression of proinflammatory cytokines and MMPs. Thus UV-B irradiation results in epidermal hyperplasia and decreased collagen in the dermis through altered signal transduction and the transcription of relevant genes.

Ingested collagen is digested and absorbed in the digestive tract and appears in the blood partly in a peptide form.[7] Possible explanations for the effect of collagen peptide are as follows: first, antioxidative activity, and second, other biological activity of the CP-derived peptide.

The effects of ingested CP on UV-B-induced skin damage observed in this study may be due to the antioxidative activity of this CP-derived peptide, as CP was found to be antioxidative *in vitro* though it should be elucidated whether the concentration of this CP-derived peptide in the skin is high enough to exhibit antioxidative activity.[8] On the other hand, it was reported that the peptide-form CP in the blood contains oligopeptides such as prolyl-hydroxyproline (Pro-Hyp).[9] It had been reported that Pro-Hyp has a chemotactic activity for cultured fibroblasts.[10] Although it is still unclear whether Pro-Hyp mediates the function of ingested collagen, it is tempting to speculate that Pro-Hyp affects the signal transduction pathway of epidermal keratinocyte and/or dermal fibroblasts and antagonizes the effect of UV-B irradiation. In that event, Pro-Hyp might directly affect the function of epidermal cells. It is also possible that Pro-Hyp affects the function of dermal cells and consequently alters the phenotype of keratinocyte, because the function of the keratinocyte can be regulated by dermal cells.[11] In any case, *in vivo* distribution of the oligopeptide and its biological activities calls for further study.

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