Photodermatology, Photoimmunology & Photomedicine

ORIGINAL ARTICLE

Collagen hydrolysate intake improves the loss of epidermal barrier function and skin elasticity induced by UVB irradiation in hairless mice

Chisato Oba, Hiroki Ohara, Masashi Morifuji, Kyoko Ito, Satomi Ichikawa, Keiko Kawahata & Jinichiro Koga

Food Science Research Labs, Meiji Co., Ltd, Odawara-shi, Kanagawa, Japan.

Key words:
collagen hydrolysate; skin barrier function; skin elasticity; stratum corneum; transepidermal water loss

Correspondence:
Ms Chisato Oba, M. Sc., Food Science Research Labs, Meiji Co., Ltd, 540 Naruda, Odawara-shi, Kanagawa 250-0862, Japan.
Tel: +81 465 37 3652
Fax: +81 465 37 3638
e-mail: chisato.ohba.ba@meiji.com

Accepted for publication:
31 May 2013

Conflicts of interest:
Nitta Gelatin Inc. is one of the suppliers of food ingredients for our products.

SUMMARY

Background

Ultraviolet B (UVB) irradiation induces serious damage to the skin. Collagen hydrolysate and collagen-derived peptides have effects on skin function *in vivo* and *in vitro*. However, few studies have investigated changes in the epidermal barrier or dermal elasticity caused by UVB. Here, we investigated the loss of epidermal barrier function and skin elasticity induced by UVB irradiation in hairless mice fed collagen hydrolysate.

Methods

Mice were orally administered collagen hydrolysate, in a single dose (20 mJ/cm^2) or repeated doses (10–30 mJ/cm^2, 3 times/week for 6 weeks), and the dorsal skin was exposed to UVB. Skin measurements and histological and analytical studies were performed.

Results

In control mice, a single UVB irradiation induced epidermal barrier dysfunction including an increase in transepidermal water loss (TEWL), epidermal hyperplasia, and a decrease in stratum corneum water content. Administration of collagen hydrolysate significantly decreased TEWL and epidermal thickness and increased stratum corneum water content. Repeated UVB irradiation decreased skin elasticity and dermal hyaluronic acid (HA) content in control mice, whereas collagen hydrolysate significantly suppressed both the increase in TEWL and the decrease in stratum corneum water content and improved skin elasticity and dermal HA content.

Conclusions

Collagen hydrolysate administration affects epidermal barrier function and dermal skin elasticity.

Photodermatol Photoimmunol Photomed 2013; 29: 204–211

10.1111/phpp.12051

Ultraviolet B (UVB) irradiation is the major environmental factor that affects the structure and function of the skin. Transient strong UVB stimulation initiates an inflammatory response in the epidermis, resulting in the induction of rough skin and barrier dysfunction (1). Long-term exposure to UVB radiation, called photoaging, damages both the dermal and epidermal skin and leads to laxity (2), wrinkling (3), thickening (4), and pigmentation (5).

Dietary supplements have demonstrated beneficial effects on skin health. Oral supplementation with food ingredients, such as vitamins and polyphenols, helps modulate skin function (6–8). Collagen is well known as a major constituent of connective tissues, such as the dermis, bone, cartilage, and tendons. Gelatin, a denatured form of collagen that is prepared from animals, birds, and fish on an industrial scale (9), is popularly used in foods. Collagen hydrolysate is manufactured by the hydrolysis of gelatin with a protease. Recent reports have shown that collagen-derived peptide, which is detected in blood after the ingestion of collagen hydrolysates (10, 11), stimulates chemotaxis (12), cell proliferation (13), and expression of the hyaluronic acid (HA) synthase gene (14) in human dermal fibroblasts. In addition, collagen hydrolysate intake increases skin collagen expression and suppresses matrix metalloproteinase 2 activity in normal rats (15). These findings led us to speculate as to whether intake of collagen hydrolysate may have a beneficial effect on UVB-induced decrease in epidermal barrier function and skin elasticity. Tanaka *et al.* (16) recently reported that collagen peptide ingestion suppressed decrease in skin hydration and increase in epidermal thickness after repeated UVB irradiation of the skin of hairless mice. However, there is only limited information on whether collagen hydrolysate intake reduces UVB irradiation-induced loss of epidermal barrier function and skin elasticity in humans or animals. Therefore, the objective of the present study was to examine the effect of collagen hydrolysate intake on transepidermal water loss (TEWL), stratum corneum water content, and skin elasticity after either a single dose or repeated doses of UVB irradiation in hairless mice.

METHODS

Animals

Nine-week-old female Hos:HR-1 hairless mice (Nippon SLC Inc., Shizuoka, Japan) were used in this study. All mice were housed in plastic cages (four mice/cage) in a temperature- and humidity-controlled room (24 ± 1°C and 50 ± 10% humidity) under a 12-h light–dark cycle. Mice were allowed free access to a standard AIN-93G diet (Oriental Yeast Co., Ltd, Tokyo, Japan) and water. All of the animal experiments in this study were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by Meiji Co., Ltd.

Experiment 1 (single dose of UVB radiation)

The experimental design followed a procedure modified from Hirotsune *et al.* (17) and Takagi *et al.* (18). Twenty-four mice were randomly divided into three groups ($n = 8$/group) according to body weight, TEWL, and stratum corneum water content. All three groups were given deionized water at 10 ml/kg body weight, and two groups were additionally given protein hydrolysate at 2.0 g/kg body weight; one of these was given casein hydrolysate as the control protein hydrolysate, and the other was given fish scale collagen hydrolysate. Fish scale collagen hydrolysate was purchased from Nitta Gelatin Inc. (Osaka, Japan). Casein hydrolysate was hydrolyzed to approximately uniform molecular weight using protease. Mice were given the experimental diet orally for 11 days, from 1 week before UVB irradiation (day -7) until 4 days after irradiation (day 4). At 1 week after initiation of the experimental diet, the dorsal skin was exposed once to 20 mJ/cm^2 of UVB (GL20SE, Sankyo Denki Co., Ltd, Tokyo, Japan) under isoflurane anesthesia. TEWL and stratum corneum water content were measured 7 days before and 0, 1, 2, 3, and 4 days after irradiation.

Another set of mice were also administered water or collagen hydrolysate as described above. Four days after irradiation, all mice were sacrificed, and the dorsal skin was excised quickly and fixed in 10% neutral buffered formalin solution for at least 24 h for histological analysis.

Experiment 2 (repeated doses of UVB radiation)

Twenty mice were allowed free access to food and water for 7 weeks. Mice were assigned randomly to two groups ($n = 10$/group) according to their body weight, TEWL, stratum corneum water content, and skin elasticity. The control group was fed the control diet (AIN-93G), and the collagen group was fed the collagen diet (a mixture containing 2 g of collagen hydrolysate per 100 g of the control diet). Fish scale collagen hydrolysate was purchased from Nitta Gelatin Inc. The composition of the experimental diets is shown in Table 1. Food intake and body weight were measured once weekly for all groups. After the mice had received 1 week of their assigned diet, the dorsal skin of each was exposed to UVB irradiation three times a week for 6 weeks. The doses of UVB per irradiation were increased gradually (19, 20). The dose was set at 10 mJ/cm^2

Table 1. Composition of the experimental diets

	Control group (%)	Collagen group (%)
Casein	20.0	20.0
L-Cystine	0.3	0.3
Collagen hydrolysate	–	2.0
Cornstarch	39.75	39.75
Pregelatinized cornstarch	13.2	13.2
Sucrose	10.0	10.0
Cellulose powder	5.0	5.0
Soybean oil	7.0	7.0
Mineral mixture[1]	3.5	3.5
Vitamin mixture[1]	1.0	1.0
Choline bitartrate	0.25	0.25
tert-Butylhydroquinone	0.0014	0.0014
	100.0	102.0

[1]Reeves *et al.* (30)

for the first week, 15 mJ/cm^2 for the second week, 20 mJ/cm^2 for the third week, and 30 mJ/cm^2 for the remaining weeks. TEWL, stratum corneum water content, and skin elasticity were measured each week. At 6 weeks after initiation of UVB irradiation, all mice were sacrificed, and the dorsal skin was excised quickly and stored at -80°C until analysis.

Measurement of TEWL and stratum corneum water content

TEWL and stratum corneum water content were assessed under standardized conditions (external temperature 24 ± 1°C and humidity 50 ± 10%). TEWL and stratum corneum water content were measured with a Tewameter MPA580 (Courage and Khazaka Electronic GmbH, Cologne, Germany) and a SKICON 200-EX skin surface hygrometer (IBS Co., Shizuoka, Japan), respectively.

Measurement of skin elasticity

Skin elasticity was recorded with a Cutometer SEM575 (Courage and Khazaka) as described previously (19). The kinetics of skin displacement (2-mm-diameter probe) in response to 2 s of 300-mbar suction followed by a 2-s relaxation period were measured. The key parameter of skin elasticity (R2, total recovery from deformation divided by total deformation) was calculated from the distension kinetics. Measurements were performed in triplicate.

Histological analysis and measurement of epidermal thickness

The dorsal skin sections were stained with hematoxylin and eosin (H&E). The thickness of the epidermis (the distance from the bottom of the basal layer to the top of the granular layer) was measured with a BX-2 biomicroscope (Olympus, Tokyo, Japan) and a DP-72 CCD camera (Olympus). It was digitally assessed by image measurement and analysis with WinROOF software (Mitani Corporation, Tokyo, Japan). The average of 20 random determinations was considered the representative value for each individual mouse.

Extraction and quantification of HA

HA was extracted based on a modified method (21, 22). The samples were treated with a solution of Dulbecco's modified Eagle's medium containing 0.1% dispase overnight at 4°C and separated into the epidermis and dermis. The separated dermis was frozen and milled in liquid N_2. The powder was recovered in 0.3 ml of a buffer containing Tris (0.1 M) and $CaCl_2$ (4 mM) (pH 8.6). The suspension was centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was decanted, and the pellet was treated with 1.2 ml of chloroform/methanol (2 : 1) and shaken gently for 30 min at room temperature. The suspension was centrifuged at 10 000 *g* for 20 min at 4°C, and the chloroform layer was extracted. This extraction was repeated twice. The defatted skin was vacuum-dried for 3 h at 30°C, and 1 ml of protease from *Streptomyces griseus* (Sigma-Aldrich Japan, Tokyo, Japan) was added. This solution was incubated for 24 h at 50°C and boiled for 10 min at 100°C. The suspension was centrifuged at 14 000 *g* for 10 min at 4°C. The supernatant was stored at -80°C until analysis. Dermal HA quantification was performed by enzyme-linked immunosorbent assay with a hyaluronic acid measurement kit (Seikagaku Corp., Tokyo, Japan).

Statistical analysis

All data are presented as mean ± standard error. Differences in the data between time zero and each time point were analyzed with Student's paired *t*-test (SPSS 14.0J, SPSS Inc., Chicago, IL, USA). Differences in the data for the three groups were analyzed by one-way ANOVA, with post hoc analyses being carried out using Tukey's test, while comparisons between the control and collagen groups were performed with Student's *t*-test. Differences were considered to be significant at $P < 0.05$.

Photodermatol Photoimmunol Photomed 2013; 29: 204–211

RESULTS

Experiment 1

TEWL and stratum corneum water content are shown in Fig. 1a and b. TEWL was significantly higher in the control and collagen groups on days 1, 2, 3, and 4 after irradiation than on day 0. A significant increase in TEWL was observed in the casein group on days 2, 3, and 4 compared with day 0. TEWL decreased in the collagen group as compared with the control group on days 2, 3, and 4. A significant increase in TEWL was observed in the casein group on days 3 and 4 as compared with the collagen group. The stratum corneum water content was significantly lower in all groups on days 1, 2, 3, and 4 after irradiation than on day 0. Collagen hydrolysate administration caused a significant increase in the water content as compared with control and casein on days 1, 2, 3, and 4.

H&E-stained dorsal skin sections and epidermal thickness 4 days after UVB irradiation are shown in Fig. 2a and b. Remarkable parakeratosis (indicated by the arrow in Fig. 2a), thickening of the prickle-cell layer, and an increase of epidermal cells were observed only in the control group. Epidermal thickness was significantly lower in the collagen group as compared with the control group.

Experiment 2

Body weight and food intake were similar between groups (data not shown). Each mouse in the collagen group was fed 2.56 ± 0.03 g/kg body weight/day of collagen hydrolysate.

TEWL and stratum corneum water content are shown in Fig. 3a and b. TEWL was significantly higher in the control group at weeks 2, 4, and 6 than at week 0. A significant increase in TEWL was found in the collagen group at weeks 4 and 6 compared with week 0. TEWL was significantly lower in the collagen group than in the control group at weeks 4 and 6. The stratum corneum water content of both groups at all time points after initiation of

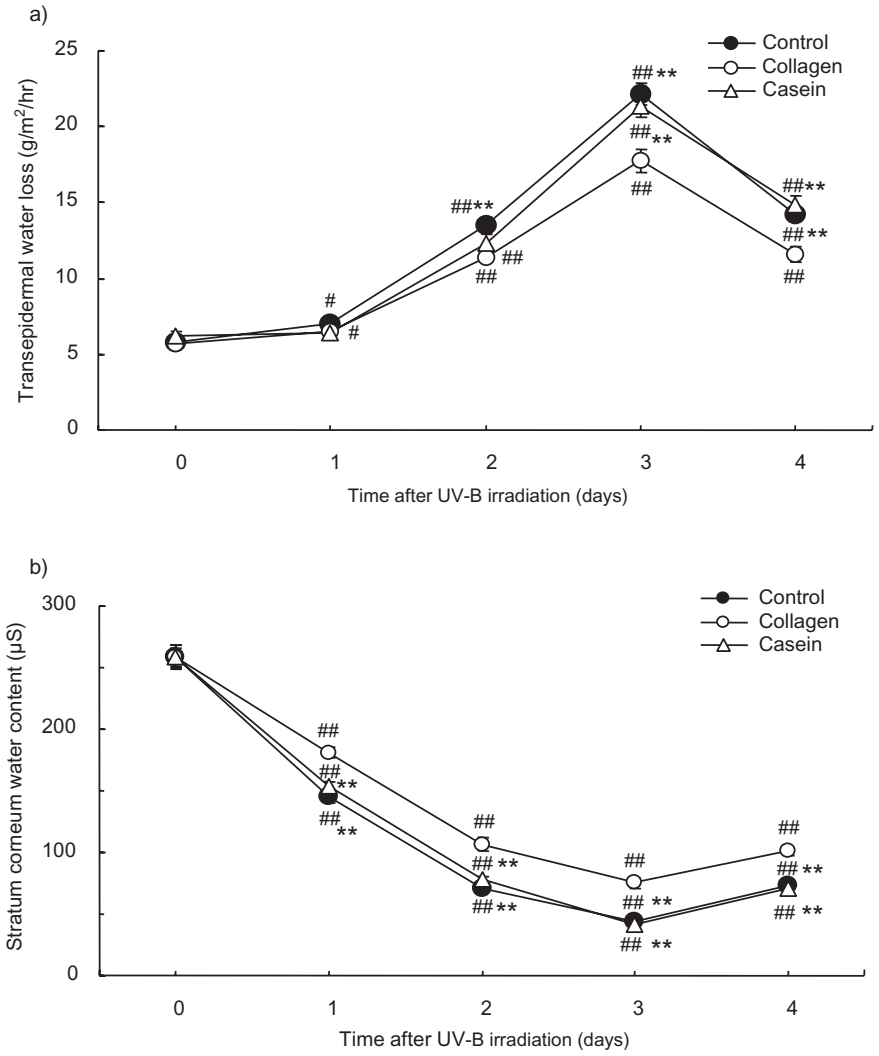

Fig. 1. Effects of collagen hydrolysate on transepidermal water loss (a) and stratum corneum water content (b) after a single dose of UVB irradiation. The values are shown as mean ± SEM ($n = 8$). $^{**}P < 0.01$ (vs. the collagen group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ (vs. day 0).

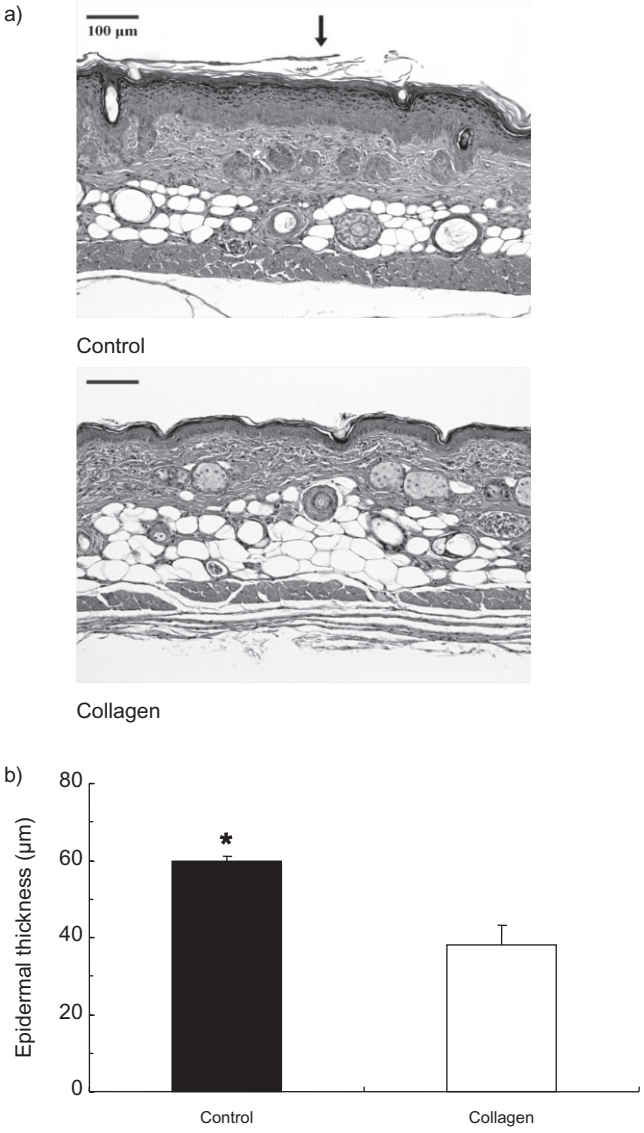

Fig. 2. H&E-stained dorsal skin sections (a) and epidermal thickness (b). The values of epidermal thickness 4 days after UVB irradiation are shown as mean ± SEM ($n = 8$). *$P < 0.05$ (vs. the collagen group).

UVB irradiation was significantly lower than at week 0. Collagen ingestion significantly increased the water content at weeks 2, 4, and 6.

Skin elasticity is shown in Fig. 3c. The R2 value was significantly lower in the control group at all time points after initiation of irradiation than at week 0, while the value in the collagen group at week 2 was significantly lower than at week 0. Skin elasticity increased significantly in the collagen group compared with the control group at week 6. Dermal HA content 6 weeks after initiation of UVB irradiation is shown in Fig. 4. HA content was significantly higher in the collagen group than in the control group.

DISCUSSION

This study evaluated the effect of collagen hydrolysate ingestion on skin damaged by UVB irradiation, especially on the epidermal barrier and on dermal elasticity defects, in hairless mice. We showed for the first time that the ingestion of collagen hydrolysate significantly reduced both epidermal barrier and skin elasticity abnormalities induced by UVB irradiation.

In this study, a single dose of UVB irradiation significantly increased TEWL and decreased stratum corneum water content. In addition, in this animal model, epidermal hyperplasia was observed 4 days after a single dose of UVB irradiation. Haratake *et al.* (23) also observed a barrier alteration 72 h after UVB irradiation in hairless mice. This delay in the barrier abnormality depends on cellular or metabolic changes in underlying skin layers. This earlier study also showed that UVB exposure increases DNA synthesis and prostaglandin E2 levels as a consequence of epidermal hyperplasia. Furthermore, the UVB-induced barrier defect was linked to a hyperproliferative response in epidermal keratinocytes. In our study, administration of collagen hydrolysate significantly suppressed not only the increase in TEWL and decrease in the stratum corneum water content after a single dose of UVB irradiation but also the resulting epidermal hyperplasia, although Tanaka *et al.* (16) found the same effects after repeated irradiation. It is possible, therefore, that intake of collagen hydrolysate suppresses UVB irradiation-induced proliferative responses in the stratum corneum, although the mechanism of this action remains unclear.

Furthermore, the current study also demonstrated that ingestion of the collagen hydrolysate reduced the loss of epidermal barrier function and skin elasticity induced by repeated UVB irradiation and increased the HA content in dermis. It is possible that dermal HA may have an important role in both epidermal barrier function and skin elasticity.

Skin elasticity is affected by repeated UVB exposure. Chronic UVB exposure can result in alterations in dermal structure and elasticity (19, 24). Elasticity is closely associated with the dermal components, especially the extracellular matrix. The extracellular matrix is a dense meshwork of collagen and elastin, embedded in a viscoelastic ground substance composed of proteoglycans and glycoproteins, such as HA (25). It has been shown that exposure of skin to chronic UVB irradiation damages the basement membrane and decreases the dermal HA content, owing to the suppression of mRNA expression of HA synthase (26). Therefore, increasing dermal HA content may reduce the

Photodermatol Photoimmunol Photomed 2013; 29: 204–211

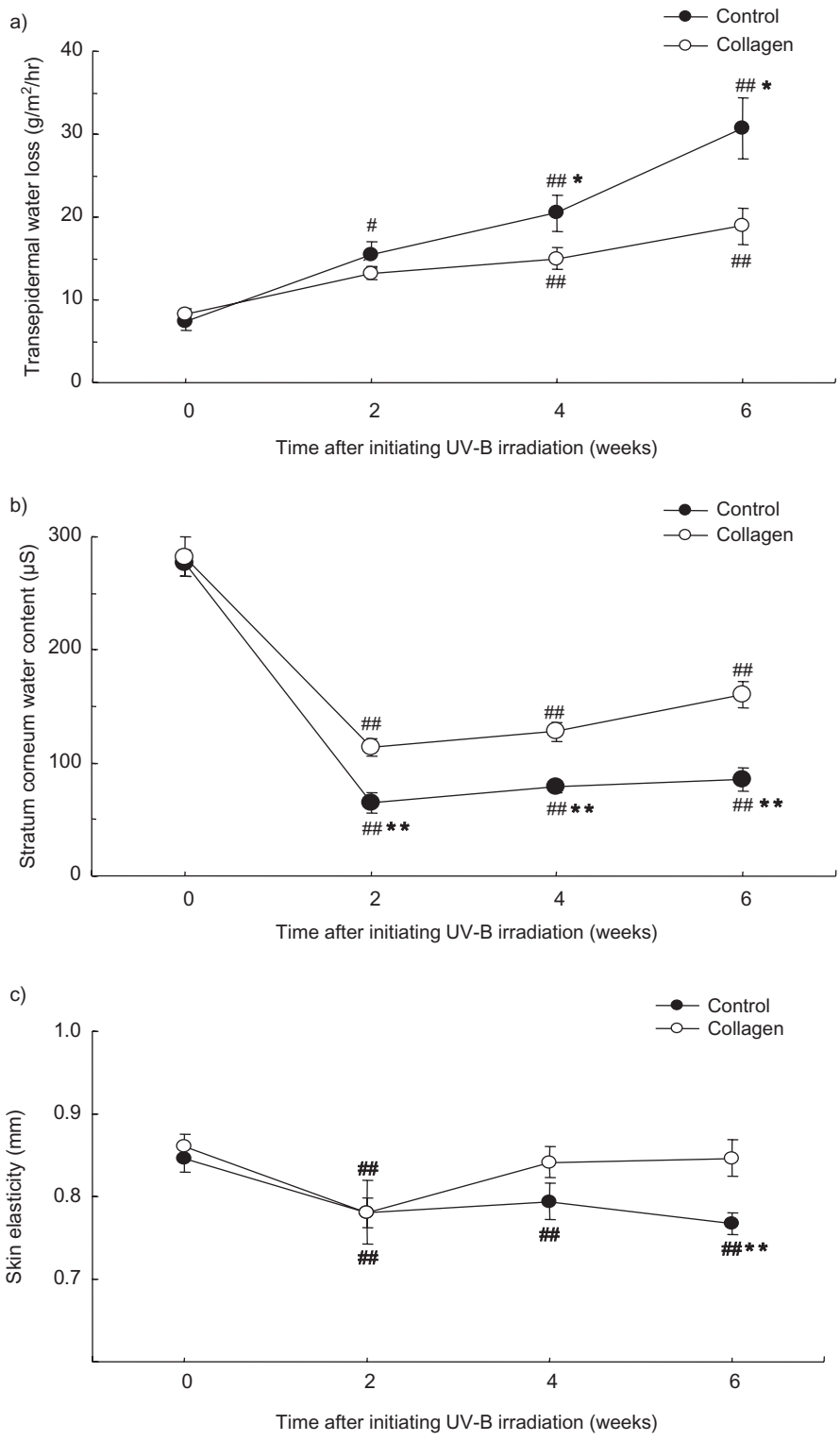

Fig. 3. Effect of collagen hydrolysate on transepidermal water loss (a), stratum corneum water content (b), and skin elasticity (c) after repeated irradiation with UVB. The values are shown as mean ± SEM ($n = 10$). *$P < 0.05$, **$P < 0.01$ (vs. the collagen group). #$P < 0.05$, ##$P < 0.01$ (vs. day 0).

UVB-induced degradation of dermal matrix components, thereby increasing skin elasticity in mice fed collagen hydrolysate.

HA also acts as a kind of huge water storage system; it is essential for maintaining skin water retention as well as skin elasticity. A recent study demonstrated that exogenous HA plays a beneficial role by interacting with fibroblasts to enhance epidermal morphogenesis, improving basement membrane assembly and formation of the epidermal lipid barrier in an organotypic

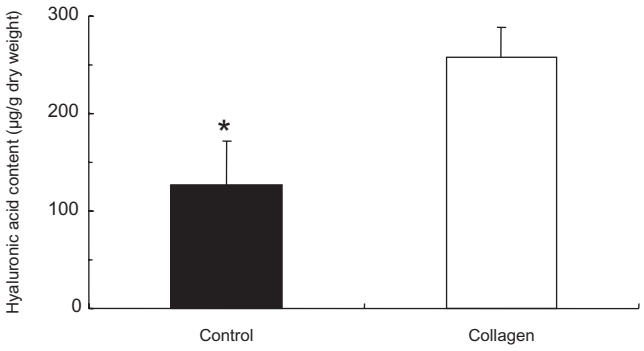

Fig. 4. Hyaluronic acid content in the dermis 6 weeks after initiation of UVB irradiation, shown as mean ± SEM ($n = 10$). *$P < 0.05$ (vs. the collagen group).

keratinocyte–fibroblast coculture model (27). Okawa *et al.* (28) also reported that collagen hydrolysate intake increased the expression of HAS as a consequence of a reduction in acetone-induced skin dryness in model mice. Thus, an increase in dermal HA may have a beneficial role in epidermal homeostasis, resulting in an increase in stratum corneum water content.

It is known that collagen has a high concentration of hydroxyproline compared with other dietary protein sources. Our previous reports showed that not only amino acids but also di- and tripeptides, such as prolyl-hydroxyproline (Pro-Hyp), alanyl-hydroxyproline, and prolyl-hydroxyprolyl-glycine, were detected in human blood after the intake of collagen taken together with hydrolysate (10, 11). The major dipeptide, Pro-Hyp, induced cell proliferation and HA synthesis in human dermal fibroblasts (14). Maximal stimulation of cell proliferation and hyaluronan synthesis by Pro-Hyp was achieved at doses of 200 nmol/mL, which is similar to physiological concentrations because the concentration of collagen-derived hydroxyproline containing peptides in plasma 2 h after oral ingestion of collagen hydrolysate is reported to be approximately 140 nmol/mL plasma. Furthermore, our preliminary study also showed that the peak concentration of collagen-derived Hyp-containing peptides in mouse plasma was the same level as in human plasma (data not shown). In addition, orally administered C-labeled Pro-Hyp was distributed in skin partly as the intact form in rats (29). It is possible, therefore, that the active components in collagen hydrolysate stimulated dermal fibroblasts and activated signaling for HA synthesis, resulting in an increase in dermal HA content.

CONCLUSIONS

In summary, our study demonstrated that oral intake of collagen hydrolysate reduced not only skin barrier abnormalities but also skin elasticity dysfunction induced by UVB. Collagen hydrolysate administration may have a beneficial effect for the reduction of loss of epidermal barrier function and skin elasticity induced by UVB irradiation.

REFERENCES

1. Abe T, Mayuzumi J. The change and recovery of human skin barrier functions after ultraviolet light irradiation. *Chem Pharm Bull (Tokyo)* 1979; **27**: 458–462.
2. Bissett DL, Hannon DP, Orr TV. An animal model of solar-aged skin: histological, physical, and visible changes in UV-irradiated hairless mouse skin. *Photochem Photobiol* 1987; **46**: 367–378.
3. Imokawa G. Recent advances in characterizing biological mechanisms underlying UV-induced wrinkles: a pivotal role of fibrobrast-derived elastase. *Arch Dermatol Res* 2007; **300** (Suppl 1): S7–20.
4. Moon SE, Youn JI, Kim JA. The effect of ultraviolet-B exposure scheduling on the photodamage of hairless mouse skin. *Photodermatol Photoimmunol Photomed* 2000; **16**: 74–77.
5. Seidl E. On the effectiveness of different UV rays on erythema and pigmentation. *Strahlentherapie* 1963; **121**: 450–463.
6. Boelsma E, van de Vijver LP, Goldbohm RA, Klöpping-Ketelaars IA, Hendriks HF, Roza L. Human skin condition and its associations with nutrient concentrations in serum and diet. *Am J Clin Nutr* 2003; **77**: 348–355.
7. Heinrich U, Neukam K, Tronnier H, Sies H, Stahl W. Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. *J Nutr* 2006; **136**: 1565–1569.
8. Skovgaard GR, Jensen AS, Sigler ML. Effect of a novel dietary supplement on skin aging in post-menopausal women. *Eur J Clin Nutr* 2006; **60**: 1201–1206.
9. Schrieber R, Seybold U. Gelatine production, the six steps to maximum safety. *Dev Biol Stand* 1993; **80**: 195–198.
10. Ohara H, Matsumoto H, Ito K, Iwai K, Sato K. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J Agric Food Chem* 2007; **55**: 1532–1535.
11. Ichikawa S, Morifuji M, Ohara H, Matsumoto H, Takeuchi Y, Sato K. Hydroxyproline-containing dipeptides and tripeptides quantified at high concentration in human blood after oral administration of gelatin hydrolysate. *Int J Food Sci Nutr* 2010; **61**: 52–60.
12. Postlethwaite AE, Seyer JM, Kang AH. Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. *Proc Natl Acad Sci USA* 1978; **75**: 871–875.
13. Shigemura Y, Iwai K, Morimatsu F *et al.* Effect of prolyl-hydroxyproline (Pro-Hyp), a food-derived collagen peptide in human blood, on growth of fibroblasts from mouse skin. *J Agric Food Chem* 2009; **57**: 444–449.
14. Ohara H, Ichikawa S, Matsumoto H *et al.* Collagen-derived dipeptide,

proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. *J Dermatol* 2010; **37**: 330–338.
15. Zague V, de Freitas V, da Costa Rosa M, de Castro GÁ, Jaeger RG, Machado-Santelli GM. Collagen hydrolysate intake increases skin collagen expression and suppresses matrix metalloproteinase 2 activity. *J Med Food* 2011; **14**: 618–624.
16. Tanaka M, Koyama Y, Nomura Y. Effects of collagen peptide ingestion on UV-B-induced skin damage. *Biosci Biotechnol Biochem* 2009; **73**: 930–932.
17. Hirotsune M, Haratake A, Komiya A *et al.* Effect of ingested concentrate and components of sake on epidermal permeability barrier disruption by UVB irradiation. *J Agric Food Chem* 2005; **53**: 948–952.
18. Takagi Y, Nakagawa H, Yaginuma T, Takema Y, Imokawa G. An accumulation of glucosylceramide in the stratum corneum due to attenuated activity of beta-glucocerebrosidase is associated with the early phase of UVB-induced alteration in cutaneous barrier function. *Arch Dermatol Res* 2005; **297**: 18–25.
19. Tsukahara K, Nakagawa H, Moriwaki S *et al.* Ovariectomy is sufficient to accelerate spontaneous skin ageing and to stimulate ultraviolet irradiation-induced photoageing of murine skin. *Br J Dermatol* 2004; **151**: 984–994.
20. Cho HS, Lee MH, Lee JW *et al.* Anti-wrinkling effects of the mixture of vitamin C, vitamin E, pycnogenol and evening primrose oil, and molecular mechanisms on hairless mouse skin caused by chronic ultraviolet B irradiation. *Photodermatol Photoimmunol Photomed* 2007; **23**: 155–162.
21. Margelin D, Medaisko C, Lombard D, Picard J, Fourtanier A. Hyaluronic acid and dermatan sulfate are selectively stimulated by retinoic acid in irradiated and nonirradiated hairless mouse skin. *J Invest Dermatol* 1996; **106**: 505–509.
22. Tobiishi M, Sayo T, Yoshida H *et al.* Changes in epidermal hyaluronan metabolism following UVB irradiation. *J Dermatol Sci* 2011; **64**: 31–38.
23. Haratake A, Uchida Y, Schmuth M *et al.* UVB-induced alterations in permeability barrier function: roles for epidermal hyperproliferation and thymocyte-mediated response. *J Invest Dermatol* 1997; **108**: 769–775.
24. Inomata S, Matsunaga Y, Amano S *et al.* Possible involvement of gelatinases in basement membrane damage and wrinkle formation in chronically ultraviolet B-exposed hairless mouse. *J Invest Dermatol* 2003; **120**: 128–134.
25. Laurent TC, Fraser JR. Hyaluronan. *FASEB J* 1992; **6**: 2397–2404.
26. Dai G, Freudenberger T, Zipper P *et al.* Chronic ultraviolet B irradiation causes loss of hyaluronic acid from mouse dermis because of down-regulation of hyaluronic acid synthases. *Am J Pathol* 2007; **171**: 1451–1461.
27. Gu H, Huang L, Wong YP, Burd A. HA modulation of epidermal morphogenesis in an organotypic keratinocyte-fibroblast co-culture model. *Exp Dermatol* 2010; **19**: e336–e339.
28. Okawa T, Yamaguchi Y, Takada S *et al.* Oral administration of collagen tripeptide improves dryness and pruritus in the acetone-induced dry skin model. *J Dermatol Sci* 2012; **66**: 136–143.
29. Kawaguchi T, Nanbu PN, Kurokawa M. Distribution of prolylhydroxyproline and its metabolites after oral administration in rats. *Biol Pharm Bull* 2012; **35**: 422–427.
30. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; **123**: 1939–1951.