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Clinical Efficacy of Adiponectin-Stimulating Peptide on UV-Induced Skin Damage

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Abstract: Several studies have suggested that adiponectin is an anti-aging molecule based on its potential involvement of adipose tissue in skin aging. In this study, we investigated the anti-photoaging efficacy of an adiponectin expression-stimulating peptide derivative, pentasodium tetracarboxymethyl hexanoyl dipeptide-12 (PTHD-12), in in vitro and ex vivo human skin explant models. A double-blind, randomized, comparator placebo-controlled study was performed to confirm clinical efficacy. After irradiation with 50 mJ/cm^2 of UVB, a UV-induced decrease in adiponectin expression and an increase in inflammatory cytokines in cultured human dermal fibroblasts were prevented by the PTHD-12 treatment test peptide. Mitigation of cellular senescence and senescence-associated secretory phenotype (SASP) expressions induced by UVB (50 mJ/cm^2) exposure were also mitigated by the post-treatment of PTHD-12, which was also observed in an ex vivo human skin explant model. The restoration of filaggrin, loricrin, and claudin-1 protein expression in a cultured human skin explant was observed. A clinical study further confirmed that the restoration of UVB-induced skin damage, represented by increased skin redness and trans-epidermal water loss, was accelerated by the use of test peptide PTHD-12-containing products. These results suggest that targeting adiponectin may be a plausible strategy for the development of anti-aging ingredients.

Keywords: adiponectin; anti-aging; double-blind; randomized; placebo comparator-controlled clinical testing; skin barrier; UV-induced skin damages

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1. Introduction

Skin aging is a multifaceted process based on two major underlying intrinsic and extrinsic mechanisms. While intrinsic skin aging is associated with chronological changes and cellular senescence, extrinsic skin aging is the accumulation of environmental factors, such as ultraviolet (UV) radiation and pollution. Extrinsic aging, also referred to as photoaging, implicates the primary role of UV rays in skin aging [1]. Among the various signaling pathways involved in photoaging, transforming growth factor-β (TGF-β) signaling, mitogen-activated protein kinase (MAPK)/activator protein-1 (AP-1) signaling, nuclear factor kappa B (NF-κB), and nuclear factor erythroid 2-related factor (Nrf2) pathways have been suggested as major signaling pathways [2]. While most of these signaling mediators are released from epidermal keratinocytes and dermal fibroblasts, the potential roles of

adipose tissue in skin aging have recently been proposed [3]. In addition to the mechanical and physical changes associated with subcutaneous white adipose tissue, especially in the facial area, the physiological interaction of adipocytes with dermal fibroblasts modulating the biological activities of fibroblasts has also been reported [4].

Adipokines are biologically active peptides mainly produced by adipocytes and include leptin, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor-1 (PAI-1), and adiponectin [5,6]. However, many adipokines are also expressed and secreted by nonadipocytes. Adiponectin, an important adipokine, plays a critical role in metabolic regulation and exhibits potential protective effects in various physiological systems, including the skin [7]. While it is primarily secreted by adipose tissue, epidermal keratinocytes and dermal fibroblasts also express adiponectin receptors, AdipoR1 and AdipoR2, suggesting potential paracrine effects of adiponectin in skin cells [8]. In addition to its potential roles in cutaneous inflammatory responses [9,10] and collagen production and degradation [11], adiponectin is involved in UV-induced skin damage. Specifically, the attenuation of adiponectin expression was observed in UV-exposed skin areas, and the experimental ablation of adiponectin induces the photoaging-like symptoms of stimulating matrix metalloproteinase-1 (MMP-1) expression and inhibiting procollagen synthesis in cultured human dermal fibroblasts [12]. Similar photoprotective effects of adiponectin were observed in UVA-exposed dermal fibroblasts. An increased expression of MMP-1, MMP-3, and cyclooxygenase (COX)-2 as well as a decreased expression of type I and III collagens following UVA exposure have been partially rescued by treatment with adiponectin [13]. Despite the potential anti-UV efficacy of adiponectin, the clinical efficacy of adiponectin or adiponectin modulators in skin care has not been well studied.

Previously, we reported that heptasodium hexacarboxymethyl dipeptide-12 and its derivatives have autophagy-stimulating activities in the skin. Autophagy is a lysosome-dependent cellular recycling process essential for maintaining proper cellular homeostasis, and by stimulating autophagy signaling, various cosmetic benefits, including a reduction in cellular senescence [14] and improvements in skin barrier function [15], have been observed. Recently, we also reported that autophagy-stimulating peptide derivatives can provide soothing effects on sensitive skin [16]. Based on the close interrelationship between autophagy and adiponectin [17,18], we screened an autophagy activator library for potential adiponectin-stimulating candidates. We identified a new peptide derivative, pentasodium tetracarboxymethyl hexanoyl dipeptide-12 (PTHD-12), that stimulates adiponectin expression in dermal fibroblasts.

In this study, using an adiponectin-stimulating peptide (PTHD-12), we explored the effects of adiponectin on UV-induced skin damages using in vitro and ex vivo testing. The clinical efficacy of adiponectin-stimulating peptides in UV-exposed skin was further examined in a double-blind, randomized, placebo comparator-controlled study.

2. Materials and Methods

2.1. Materials

The test peptide pentasodium tetracarboxymethyl hexanoyl dipeptide-12 (PTHD-12) and its precursor heptasodium hexacarboxymethyl dipeptide-12 were prepared using solid-phase peptide synthesis with fluorenylmethyloxycarbonyl chloride. After synthesis, the test material was purified by reverse-phase high-performance liquid chromatography (RP-HPLC).

2.2. In Vitro Studies

Normal human dermal fibroblasts (hDFs) and the culture media used in this study were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The hDFs were maintained using a human fibroblast expansion basal medium with a low serum

growth supplement and gentamicin/amphotericin, purchased from Thermo Fisher Scientific Inc. Cells were cultured and maintained at 37 °C in a 5% CO_2 atmosphere. For a quantitative real-time polymerase chain reaction (qRT-PCR), hDFs were seeded in 6-well plates (1×10^5 cells/well) and cultured for 24 h. After removing the culture medium and washing, the cells were treated with either a serum-free medium or the test peptide PTHD-12 at a concentration of 40 ppm and further incubated for 24 h. After washing the cells with a phosphate-buffered saline solution (pH 7.4), 50 mJ/cm^2 of UVB was irradiated using a UV irradiator (CL-508, UVItec Ltd., Cambridge, UK) at the wavelength of 314 nm, and cells were further cultured for 24 h. Then, 30 ng of cDNA was prepared from the total RNA fraction of cell lysates, as described previously [19], and PCRs were performed using the QuantStudio™ 3 system (Thermo Fisher Scientific Inc.), according to the manufacturer's instructions. The following primer sets were used for quantitation: adiponectin, 5′-CAGATGCCCCAGCAAGTGTA-3′ and 5′ 5′-TCAGAAACAGGCACACAACTCA-3′; MMP-1, 5′- CTCGCTGGGAGCAAACACA-3′ and 5′-CTTGGCAAATCTGGCGTGTA-3; and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-GGAGTCAACGGATTTGGTCGTA-3′, and 5′-GCAACAATATCCACTTT ACCAGAGTTAA-3′. Gene expression levels were calculated as cycle threshold (Ct) values using the $2^{-\Delta\Delta CT}$ quantification method and normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

To measure protein quantity, hDFs were seeded in 6-well plates (2×10^4 cells/well) and cultured for 24 h. After removing the culture medium and washing, the cells were treated with the test peptide PTHD-12 for 24 h, and cell lysates were harvested. The cell lysates were then separated by SDS-PAGE, and proteins were blotted onto nitrocellulose membranes. After blocking with a 0.5% bovine serum albumin solution containing 0.1% Tween-20, the membranes were incubated with primary antibodies overnight at 4 °C. Secondary antibodies conjugated with horseradish peroxidase were incubated for one hour at room temperature, and protein quantity was measured using an ECL detection reagent (Amersham Biosciences Corp., Little Chalfont, UK) using the ChemiDoc system (Alliance Mini HD 9, Uvitec, Cambridge, UK).

To observe the adiponectin expression in dermal fibroblasts, the cells were seeded on a 2-well cell culture slide and cultured for 24 h. After UVB exposure and sample treatment, cells were fixed with a 4% paraformaldehyde solution for 20 min. After rinsing, the cells were permeabilized with 0.1% Triton X-100 in a PBS solution for 10 min at room temperature and treated with a 5% bovine serum albumin solution for 60 min. The cells were then incubated with a mouse anti-human adiponectin antibody (ab22554; Abcam, Cambridge, MA, USA) overnight at 4 °C. After rinsing, the cells were treated with goat anti-mouse IgG H&L (Alexa Fluor® 568, ab175473, Abcam) for 60 min at room temperature and examined under a conformal LASER scanning microscope (LSM 880 with Airyscan, Carl Zeiss, Oberkochen, Germany).

The expression of interleukin-8 (IL-8) was measured using an ELISA kit (R&D Systems, Cat. No. DY208, Minneapolis, MN, USA), according to the manufacturer's instructions, as previously described [20].

2.3. Ex Vivo Studies

A NativeSkin® ex vivo human skin explant model was procured from GeneSkin SAS (Toulouse, France). A 24-year-old female Caucasian donor with a type 3 Fitzpatrick skin type, with no history of allergy aordermatological disorders, underwent abdominoplasty, and anonymized human skin samples were retrieved. Ethical compliance was enacted, authorized through the official authorization from the French Ministry of Research (Protocol AC-2022-4863, 14 October 2022), and the study was performed in accordance with the

principles of the Declaration of Helsinki. After surgery, the skin tissues were harvested, and the subcutaneous adipose tissues were carefully excised from the skin. Punch biopsies that were 11 mm in diameter were taken, and tissues were embedded in a proprietary biological matrix in Transwell inserts (Millicell, Merck KGaA, Darmstadt, Germany), following the proprietary protocol developed by Genoskin. The epidermal surface of the skin tissue was maintained in direct contact with the air, and the dermal side was immersed in the biological matrix. The tissue was cultured in a chemically defined, serum-free medium with 100 μg/mL of penicillin and 100 μg/mL of streptomycin and cultivated in a humidified chamber with 5% CO_2 at 37 °C. After receiving the tissues, the skin models were stabilized in a 12-well culture plate containing 1 mL of a maintenance medium (Genoskin) in a 5% CO_2 humidified chamber. After 2 h of stabilization, the culture medium was replaced with a fresh maintenance medium and maintained until the next step. After irradiation with 50 mJ/cm^2 of UVB on the epidermal surface of the skin tissue, either 25 μL of the test sample solution or a blank solution was topically applied onto the skin surface once a day for 2 days. At 24 h after the second application, tissues were harvested, fixed with a 4% neutral buffered formalin solution overnight at 4 °C, and embedded in paraffin. For immunohistochemical staining of the tissue, 5μM-thick tissue sections were deparaffinized and rehydrated before heat-induced epitope revival treatment in an antigen retrieval solution (ab937, Abcam) at pH 6.0 for 10 min at 97 °C, followed by cooling for 10 min in a cooling chamber. After blocking non-specific antibody binding using a blocking reagent (X0909, Agilent, Santa Clara, CA, USA), a mouse anti-human anti-adiponectin (Abcam, ab22554) antibody was applied to the tissue and incubated in a humidified chamber overnight at 4 °C. The epidermal expression of interleukin-6 and tumor necrosis factor-α was observed using a mouse anti-human TNF-α antibody (ab9579, Abcam) and a rabbit anti-human IL-6 antibody (ab6672, Abcam), respectively. Either goat anti-rabbit immunoglobulin (IgG) H&L (Alexa Fluor® 488, ab150077, abcam) or goat anti-mouse IgG H&L (Alexa Fluor® 568, ab175473, abcam) were added to the tissues, and fluorescence intensity was analyzed under a fluorescence microscope (Eclipse Ni-U, Intenslight C-HGFI, DS-Ri2, Nikon, Tokyo, Japan) at 400× magnification. All experiments were performed in triplicate.

2.4. Clinical Efficacy Testing

To assess the clinical efficacy of the test peptide PTHD-12 against UV-induced skin damage, a double-blind, randomized, placebo comparator-controlled study was performed. A total of 20 healthy female volunteers (46.7 ± 4.4 years old: mean ± standard deviation) were enrolled. The following exclusion criteria were adopted to select participants.

- Pregnant or lactating women, and women with a potential for pregnancy;
- Individuals who used topical steroid-containing dermatological drugs within the past one month for skin disease treatment;
- Participants who have engaged in similar clinical testing within the past 6 months;
- Individuals who have dermatological abnormalities, such as pigmentation spots, acne, erythema, and/or telangiectasia;
- Individuals who have used cosmetics or pharmaceutical products with identical or similar efficacy on the test site within the past 3 months;
- Individuals who received aesthetic procedures on the test site within the past 6 months;
- Other individuals who were considered unsuitable for testing at the principal investigator's discretion.

This study was approved by the Institutional Review Board of the Mariedm Skin Research Center (approval number: MDSRC-2400PR-168) (8th November 2024). All the studies complied with the World Medical Association's Declaration of Helsinki (2013) concerning biomedical research involving human subjects. All volunteers signed an informed

consent form and participated in the study after the purpose and protocol were explained to them. UV rays of 60.2 mJ/cm^2, which is equivalent to 2 minimal erythema dose (MED) energies, were irradiated to the dorsal areas. Before and immediately after irradiation, and at 3 days and 7 days after irradiation, trans-epidermal water loss (TEWL) and skin redness were measured in air-conditioned environments (temperature, 22 ± 2 °C; relative humidity, 50% ± 10%) after an acclimatization period of at least 30 min. TEWL, which represents the epidermal permeability barrier function, was measured using a Vapometer (Delfin, Kuopio, Finland), and skin redness was measured using a Spectrophotometer CM-2600d (Konica Minolta, Inc., Osaka, Japan). Skin images were also acquired using an Antera 3D CS (Miravex, Dublin, Ireland). After the baseline measurement, participants were administered either the test product or a placebo comparator product at randomly chosen sites twice a day, morning and night, for 7 days. The complete information regarding the test products is provided in Table 1.

Table 1. Clinical efficacy testing product information.

Test Product	Control Product
Aqua	Aqua
Propanediol	Propanediol
Diisobutyl adipate	Diisobutyl adipate
Ethylhexyl triazone	Ethylhexyl triazone
Diisopropyl sebacate	Diisopropyl sebacate
Butyloctyl salicylate	Butyloctyl salicylate
Polymethylsilsesquioxane	Polymethylsilsesquioxane
Diethylamino Hydroxybenzoyl Hexyl Benzoate	Diethylamino Hydroxybenzoyl Hexyl Benzoate
Niacinamide	Niacinamide
Drometrizole Trisiloxane	Drometrizole Trisiloxane
Methylene Bis-Benzotriazolyl Tetramethylbutylphenol	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol
Caprylyl Methicone	Caprylyl Methicone
1,2-hexanediol	1,2-hexanediol
Butylene glycol	Butylene glycol
Panthenol	Panthenol
Pentylene Glycol	Pentylene Glycol
Behenyl Alcohol	Behenyl Alcohol
Polyglyceryl-3 Methylglucose Distearate	Polyglyceryl-3 Methylglucose Distearate
Polyglyceryl-6 Distearate	Polyglyceryl-6 Distearate
Decyl Glucoside	Decyl Glucoside
Tromethamine	Tromethamine
Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters	Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters
Carbomer	Carbomer
Acrylates/C10-30 Alkyl Acrylate Crosspolymer	Acrylates/C10-30 Alkyl Acrylate Crosspolymer
Sodium Stearoyl Glutamate	Sodium Stearoyl Glutamate
Polyacrylate Crosspolymer-6	Polyacrylate Crosspolymer-6
Ethylhexylglycerin	Ethylhexylglycerin
Adenosine	Adenosine

Table 1. *Cont.*

Test Product	Control Product
Glycerin	Glycerin
Xanthan Gum	Xanthan Gum
Tocopherol	Tocopherol
Pentasodium Tetracarboxymethyl Hexanoyl Dipeptide-12 (100 ppm)	-

2.5. Statistical Analysis

Values are expressed as the arithmetic mean ± standard deviation. Data normality was verified using the Shapiro–Wilk test and Kurtosis/Skewness analysis, while inter-group homogeneity was verified using one-way ANOVA and Tukey's HSD post hoc test. To compare the pre- and post-usage results and between-group differences, a Repeated Measures Analysis of Variance (RM-ANOVA) was used when normality was satisfied, and the Friedman test was used when normality was not satisfied. Statistical analyses were performed using SPSS ver. 20 (IBM, New York, NY, USA), and the significance level was set at $p < 0.05$ for all statistical results, and the improvement rate (%), based on changes before and after product use, was calculated using the mean values of all subjects' measurements, according to the following formulas.

$$\%\ Improvement\ in\ TEWL = \left(1 - \frac{TEWL_{at\ measurement\ time} - TEWL_{basal}}{TEWL_{immediately\ after\ UV\ exposue} - TEWL_{basal}}\right) \times 100$$

$$\%\ Improvement\ in\ skin\ redness = \left(1 - \frac{A\ value_{at\ measurement\ time} - A\ value_{basal}}{A\ value_{immediately\ after\ UV\ exposue} - A\ value_{basal}}\right) \times 100$$

3. Results

To address the change in adiponectin expression in dermal fibroblasts after UV exposure, cultured human dermal fibroblasts were exposed to 50 mJ/cm^2 UVB, and the mRNA and protein expression of adiponectin were measured by quantitative RT-PCR and Western blotting, respectively. As shown in Figure 1, a slight but significant reduction in both mRNA and protein levels was observed in the UVB-exposed cells. Based on a preliminary cytotoxicity assessment, the concentration of the test peptide PTHD-12 for the in vitro studies was decided as 40 ppm, which did not show any cytotoxicity. Co-treatment with the test peptide PTHD-12 restored adiponectin expression (Figure 1a,b), and confocal microscopic observations further confirmed the increased expression of adiponectin in the cytoplasmic region by the test peptide PTHD-12 (Figure 1d) compared to the non-treated cells (Figure 1c).

To investigate whether the increased expression of adiponectin could alleviate UV-induced cellular damage, the expression of inflammatory mediators was examined. As shown in Figure 2, the increased expression of matrix metalloproteinase (MMP)-1 induced by UVB (Figure 2a) and UVA (Figure 2b) was downregulated by the test peptide PTHD-12. Treatment with AdipoRon (AR), an adiponectin receptor agonist, showed a similar inhibitory activity on MMP-1 expression, suggesting the potential involvement of adiponectin signaling in UV-induced skin damage. Interestingly, treatment of normal cells with the test peptide PTHD-12 also decreased the expression of MMP-1 in dermal fibroblasts, suggesting a potential application of adiponectin-stimulating peptides for anti-wrinkle purposes. The measurement of interleukin (IL)-8, an inflammatory cytokine, in

cultured human epidermal keratinocytes further confirmed the anti-inflammatory activity of the test peptide PTHD-12 (Figure 2c).

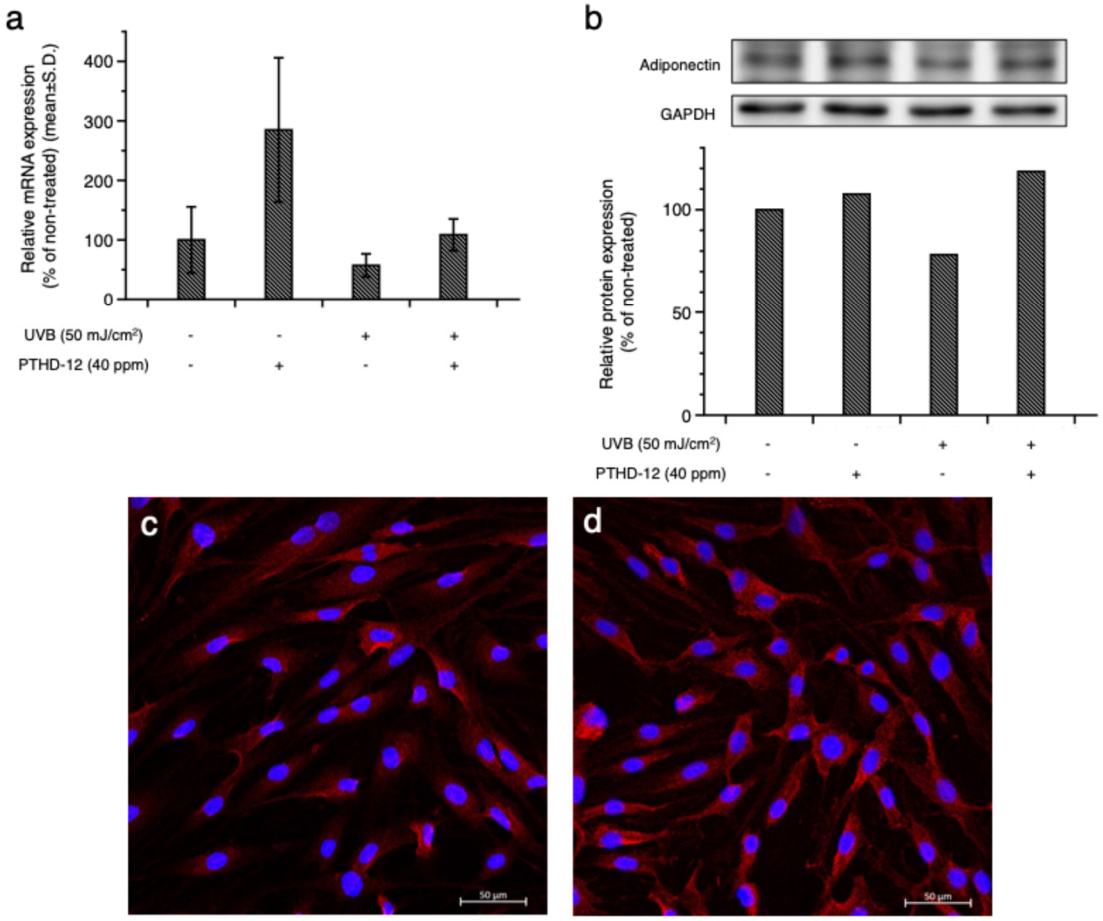

Figure 1. Stimulation of adiponectin expression by the test peptide PTHD-12 in human dermal fibroblasts. UVB exposure-induced attenuation of adiponectin expression was restored by treatment with the test peptide PTHD-12, both in mRNA and protein levels (**a**,**b**). Confocal microscopic observations further confirmed the increased expression of adiponectin by the test peptide PTHD-12 in hDF cells (**d**) compared to non-treated hDF cells (**c**). (Magnification: X400.)

Next, we evaluated the biological activity of the test peptide PTHD-12 using an ex vivo human skin explant model. Skin tissues obtained from a healthy donor were exposed to UVB rays, and changes in adiponectin and other inflammatory markers were assessed by immunohistochemical staining. As shown in Figure 3, a modest expression of adiponectin in the basal layer was observed in normal skin (Figure 3a), and UVB exposure did not result in significant changes (Figure 3b). However, the topical application of the test peptide PTHD-12 after UVB exposure significantly upregulated adiponectin expression in both basal and spinous layers (Figure 3c). UVB exposure also resulted in an increased expression of the $p16^{INK4a}$ protein (Figure 3e), a marker of senescent cells [21], compared to non-exposed tissue (Figure 3d), which is consistent with previous reports [9,22]. Interestingly, the topical application of the adiponectin-stimulating peptide also prevented the expression of $p16^{INK4a}$ after UVB exposure (Figure 3f). The epidermal expression of tumor necrosis factor-α (TNF-α) also showed similar changes, which were significantly increased by UV exposure (Figure 3h) and decreased by the application of the test peptide PTHD-12 (Figure 3i). Adiponectin upregulates the filaggrin (FLG) gene expression in cultured human epidermal keratinocytes [23]. Considering the crucial role of the filaggrin protein in skin barrier function, the beneficial effects of adiponectin

on the skin barrier were further examined. Filaggrin and loricrin were expressed in the suprabasal layer in normal tissue (Figure 4a,d) and significantly attenuated by UVB exposure (Figure 4b,e). The topical application of the adiponectin-stimulating peptide restored barrier protein expression (Figure 4c,f), and similar changes were also observed for claudin-1, which constitutes the tight junction. Collectively, these results suggest that stimulation of adiponectin expression can provide potential anti-aging and skin barrier-enhancing effects on UVB-induced photoaging.

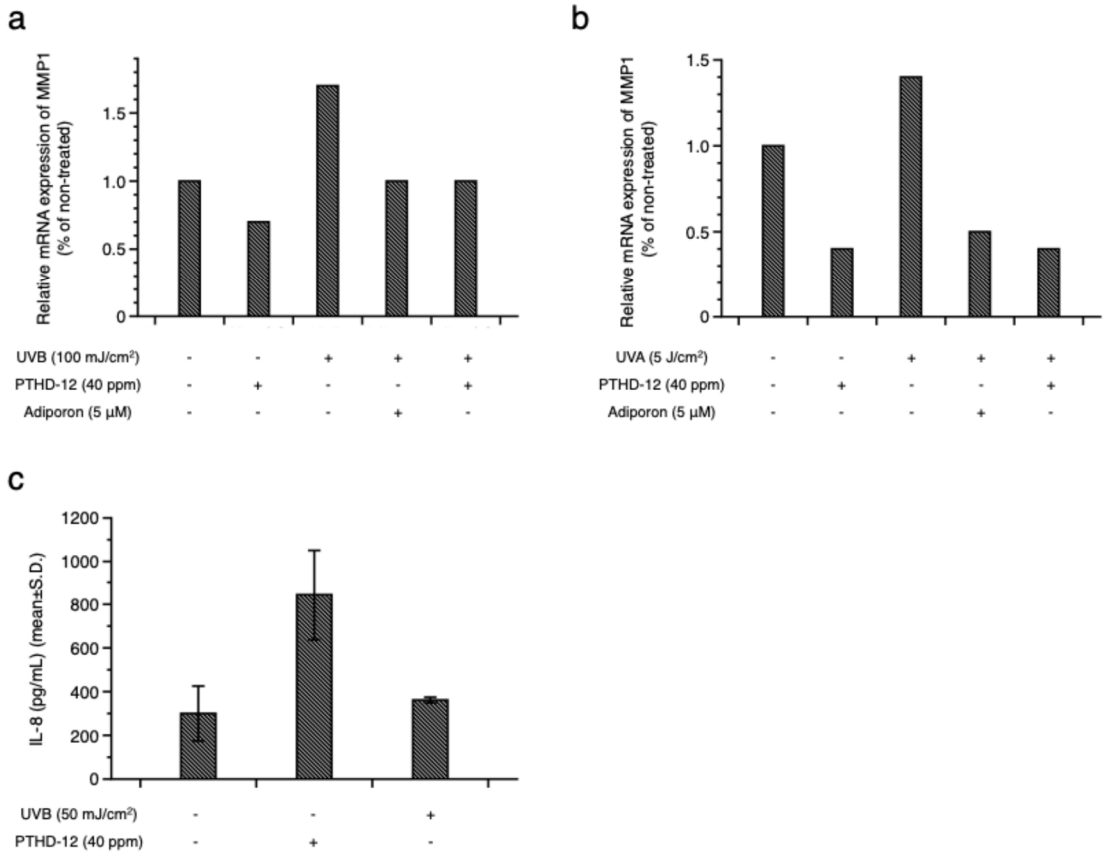

Figure 2. Alleviation of UV-induced cellular damage by adiponectin-stimulating peptide. Increased expression of MMP-1 by UVB (**a**) and UVA (**b**) was downregulated by both the test peptide PTHD-12 and AdipoRon, an adiponectin receptor agonist, in cultured human dermal fibroblasts. The increased expression of interleukin (IL)-8 in UVB-exposed human epidermal keratinocytes was also decreased by treatment with the test peptide PTHD-12 (**c**).

To verify whether the observed effects manifested clinically, a double-blind, randomized, placebo comparator-controlled clinical study was performed. Twenty healthy female volunteers (mean age, 46.7 years old ± standard deviation of 4.4 years) participated, and UVB radiation of 2 minimal erythema dose (MED) energies was irradiated on the dorsal area. Skin redness and trans-epidermal water loss (TEWL) were measured to examine their effects on skin irritation and barrier function. Significant increases in both skin redness, represented by a-values and TEWL values, were observed after UVB irradiation (Figure 5a,b). While spontaneous restoration of skin redness and skin barrier function was observed in the non-treated site, the topical application of the test product significantly accelerated the recovery rate (Figure 5c,d) compared to the placebo comparator-treated site. These results substantiate the clinical efficacy of adiponectin-stimulating peptides in UVB-induced skin damage.

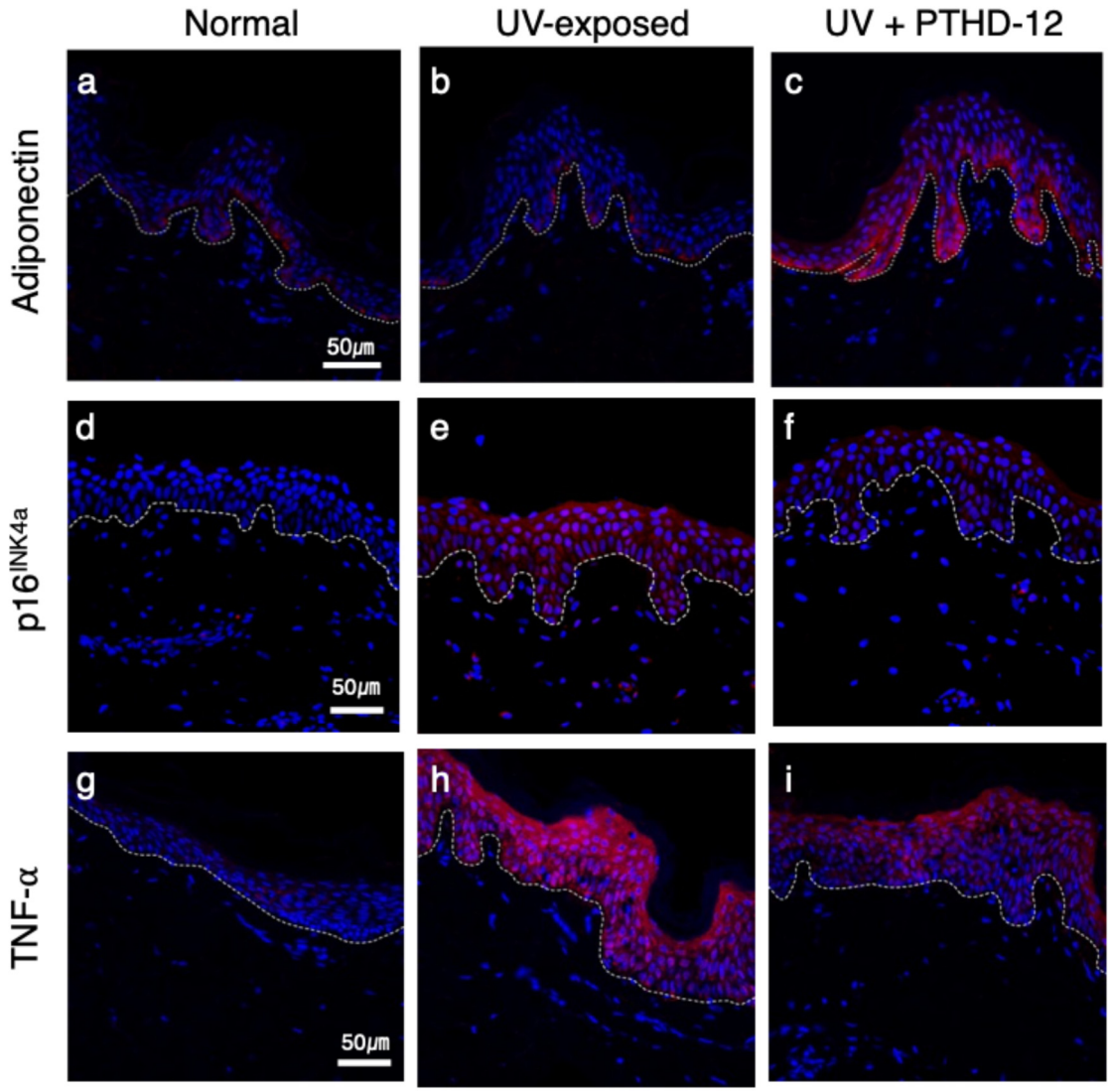

Figure 3. Stimulation of adiponectin expression by the test peptide PTHD-12 in an ex vivo human skin explant model. A significantly increased expression of adiponectin was observed in the test peptide PTHD-12-treated tissue (**c**) compared to non-irradiated (**a**) and UVB-exposed tissues (**b**). Epidermal expressions of $p16^{INK4a}$, a marker of senescence cells, were also increased by UVB exposure (**e**) compared to non-irradiated tissue (**d**), which was prevented by the application of the test peptide PTHD-12 (**f**). (Red: adiponection (**a**–**c**), $p16^{INK4a}$ (**d**–**f**), and TNF-α (**g**–**i**); blue: DAPI; dotted line: dermal–epidermal junction) (magnification: X400).

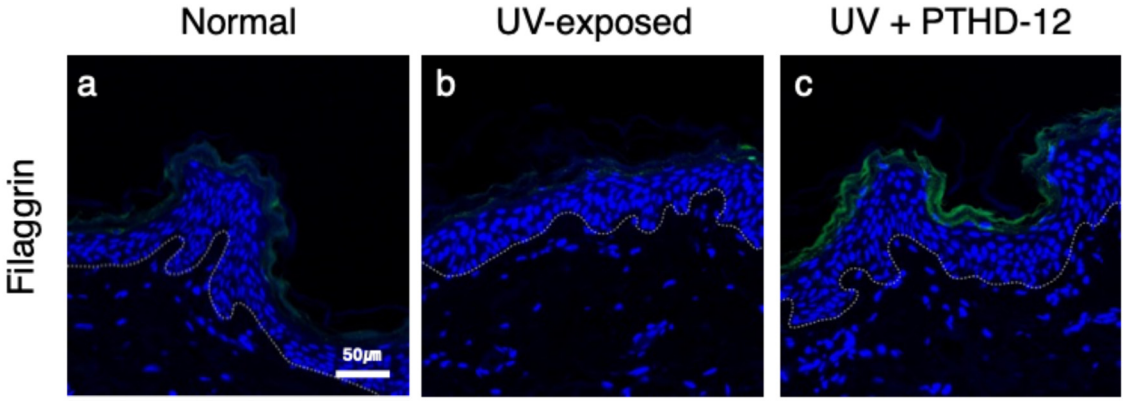

Figure 4. *Cont.*

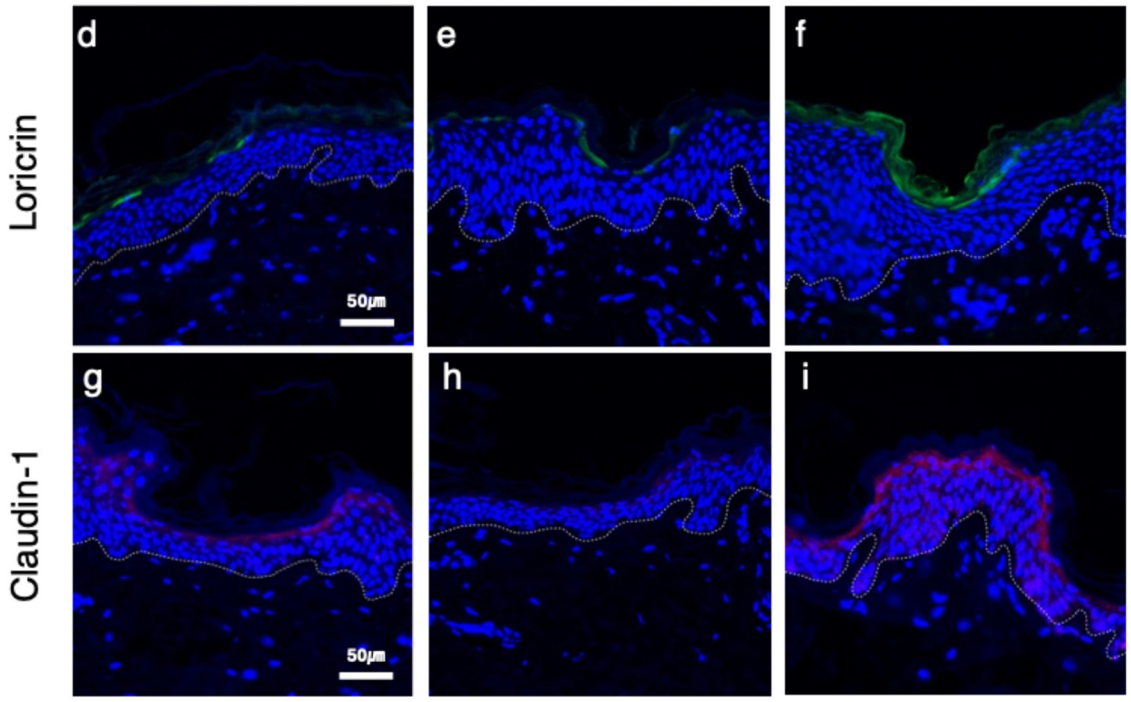

Figure 4. Restorations of skin barrier marker proteins by adiponectin-stimulating treatment of UVB-exposure-induced attenuation of protein expressions (**b**,**e**,**h**) were rescued by the application of the test peptide PTHD-12 (**c**,**f**,**i**). (Green: filaggrin (**a**–**c**), loricrin (**d**–**f**), and claudin-1 (**g**–**i**); blue: DAPI; dotted line: dermal–epidermal junction) (magnification: X400).

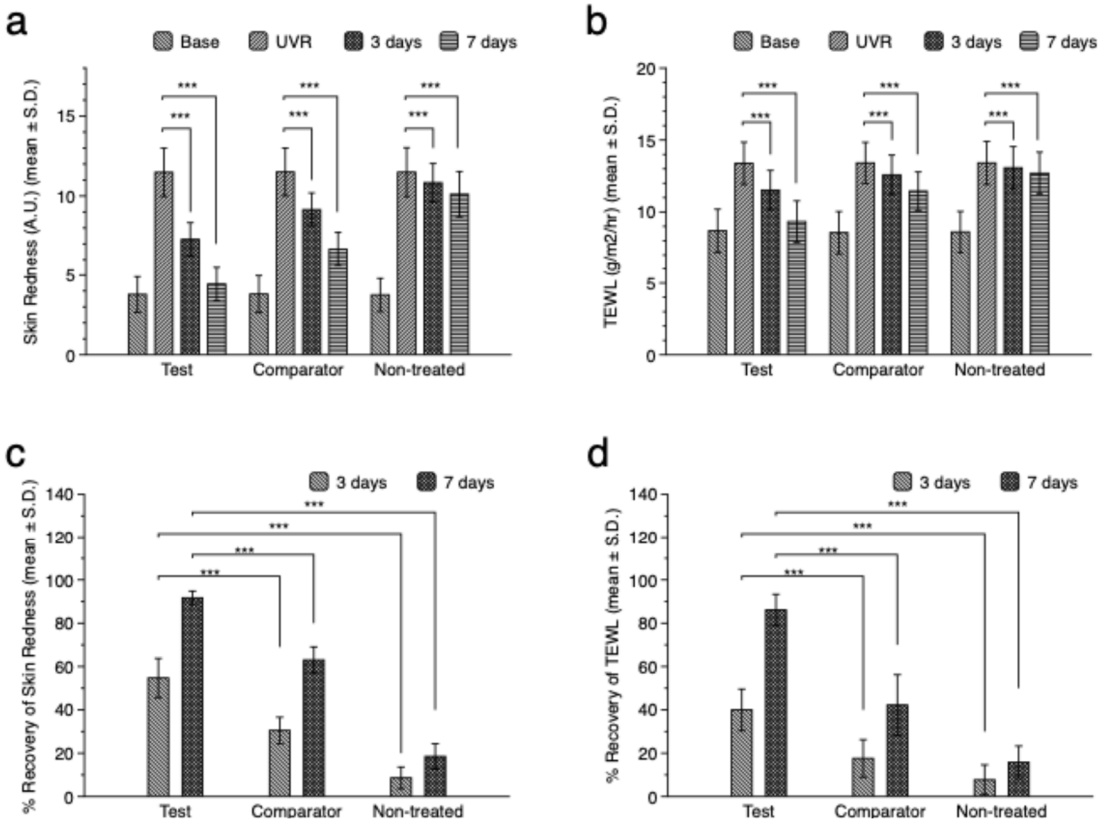

Figure 5. Changes in skin redness and trans-epidermal water loss (TEWL) after UVB irradiation. Significant increases in skin redness (**a**) and TEWL (**b**) were observed after UVB irradiation, and the topical application of either a placebo comparator product or the test product significantly accelerated the recovery rate at 3 and 7 days after irradiation (**c**,**d**). The test group showed the highest recovery rate compared with the placebo comparator and non-treated groups. (***: $p < 0.001$)

4. Discussion

Various signaling molecules, including transforming growth factor-beta (TGF-β), mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-κB), and nuclear factor erythroid 2-related factor 2 (Nrf2), have been reported to be important factors in skin photoaging. While most signaling molecules originate from either epidermal or dermal tissues, the potential involvement of adipose tissue and adipocytes in skin aging was recently proposed [24]. In addition to the important roles of adipose tissue in providing the structural integrity and mechanical properties of the skin, the signaling molecules generated from the adipose tissue, including adipokines, also play crucial roles in the regenerative capacity and immune functions of the skin [3]. Previous studies have reported that adiponectin suppresses the production of pro-inflammatory cytokines, such as interleukin (IL)-17, tumor necrosis factor (TNF)-α, and IL-6, and genetic ablation of adiponectin was shown to exacerbate inflammatory responses in an animal model of psoriasis by enhancing the infiltration of IL-17-producing T cells [25]. In this study, the potential anti-inflammatory and senopreventive activities of the adiponectin-stimulating peptide were observed. The increased expression of $p16^{INK4a}$, a well-known senescent cell marker, and TNF-α induced by UVB exposure was significantly prevented by PTHD-12 application. Similar skin-soothing activity was also observed in a clinical study. By irradiating UVB with 2 MED energies, skin irritation, represented by increased skin redness, can be induced. Use of the PTHD-12-containing product significantly accelerated the restoration of skin redness, suggesting that the stimulation of adiponectin expression can provide potential soothing effects.

Adiponectin also promotes wound healing, mainly by upregulating epidermal keratinocyte proliferation and migration [8]. The cosmetic benefits expected from adiponectin include anti-wrinkle activities by boosting the production of hyaluronic acid (HA) and collagen by dermal fibroblasts, contributing to the maintenance of the extracellular matrix and structural integrity of the skin [11]. The potential protection of UV-induced skin damage by reducing oxidative stress and inflammatory responses by adiponectin [26], which leads to the reduction in matrix metalloproteinases (MMPs), also helps to mitigate photoaging. From in vitro experiments, we also observed that UVA- and UVB-induced MMP-1 expression was prevented by treatment with the adiponectin-stimulating peptide PTHD-12. However, owing to its short testing period, we could not assess the clinical anti-aging effects of PTHD-12, which guarantees the need for further long-term studies.

Another important activity of adiponectin in the skin is the modulation of skin barrier function. Previous studies have reported that adiponectin treatment upregulated the synthesis of epidermal lipids, including ceramides [27] and filaggrin [28], which constitute the intercellular lipids and corneocytes of the stratum corneum. The stimulation of lipid and protein synthesis by adiponectin can enhance skin barrier function. In this study, we also observed an increased expression of the barrier marker proteins filaggrin and loricrin, as well as claudin-1, following PTHD-12 treatment. Claudin-1 is a major component of epidermal tight junctions, which create a dynamic paracellular barrier that regulates epidermal permeability [29]. In clinical efficacy testing, the accelerated recovery of trans-epidermal water loss, a functional parameter representing epidermal permeability barrier function, was also observed.

Although several studies have suggested the potential beneficial effects of adiponectin on skin aging, little has been reported regarding its clinical efficacy. In this study, we observed that UV-induced skin damage could be attenuated by adiponectin-stimulating peptide treatment. While this study is the first report on the clinical effects of adiponectin-stimulating peptides for cosmetic applications, it has a few limitations. First, the study was performed over a short period, and only acute effects were examined. The relatively

small number of participants also precludes drawing broad generalizations from the results. Large-scale and long-term studies are required to confirm the anti-aging effects of the test peptide PTHD-12. Additional investigations of the effects of adiponectin on intrinsic aging are necessary. However, despite its limitations, this study suggests that targeting adiponectin may be a plausible strategy for developing new skin anti-aging ingredients.

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Conflicts of Interest: Yongwoo K. was employed by the company Kolmar Korea, and S.Y., S.K., Yeonjae K. and S.J. were employed by the company Incospharm Corp. The remaining author declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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