Prev. Nutr. Food Sci. 2022;27(4):423-435
https://doi.org/10.3746/pnf.2022.27.4.423
ISSN 2287-8602

Fish Collagen Peptide (Naticol®) Protects the Skin from Dryness, Wrinkle Formation, and Melanogenesis Both *In Vitro* and *In Vivo*

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ABSTRACT: Consistent ultraviolet B (UVB) radiation exposure results in dry skin, wrinkles, and melanogenesis. In this study, we investigated whether fish collagen peptide (Naticol®) could inhibit photoaging and oxidative stress in skin exposed to UVB using cell and animal models. We measured the skin hydration, histological observations, antioxidant activities, moisturizing-related factors, collagen synthesis-related factors, and melanogenesis-related factors in skin cells and animal skin using enzyme-linked immunosorbent assay, real-time polymerase chain reaction, and Western blot assay. Naticol® collagen improved skin moisturization via hyaluronic acid and ceramide synthesis-related factors in HaCaT cells and SHK-I hairless mice that were exposed to UVB. In addition, Naticol® collagen inhibited wrinkle formation in Hs27 cells and SHK-I hairless mice exposed to UVB and restrained melanogenesis in 3-isobutyl-1-methylxanthine-induced B16F10 cells and UVB-irradiated SHK-I hairless mice. On the basis of these findings, we propose that ingestion of Naticol® collagen might be valuable for preventing skin photoaging.

Keywords: fish collagen peptide, skin health, ultraviolet B

INTRODUCTION

The skin provides physical and biochemical defense from harmful chemicals, pathogens, and ultraviolet (UV) radiation. Skin aging can be divided into two categories: intrinsic and extrinsic. Intrinsic aging occurs naturally over time and is identified by epidermal thinning, cell loss, and wrinkle formation. Extrinsic aging, or photoaging, is caused by accumulating damage from UV exposure and is identified by dyspigmentation, elasticity degradation, wrinkling, and fragility. Keratinocytes in the epidermis protect the skin by reducing the loss of heat and moisture, while other cells, such as Merkel cells, Langerhans' cells, and melanocytes, augment their function. The dermis, located under the epidermis, is composed of fibroblasts, and the extracellular matrix (ECM) is composed of glycoproteins, proteoglycans, elastin, collagen, and hyaluronic acid (HA) (Coderch et al., 2003; Tracy et al., 2016). Exposure to UV radiation leads to increased generation of reactive oxygen species (ROS) in the epidermis, which are broken down by the antioxidant defense system, resulting in oxidative stress. Oxidative stress caused by ultraviolet B (UVB) exposure contributes to a decrease in HA production, the degradation of elastin, and excessive melanin production. In addition, UVB-irradiated oxidative stress activates protein degradation and pro-inflammatory cytokine production, which lead to wrinkle formation (Rittié and Fisher, 2002; Dai et al., 2007; Cavinato and Jansen-Dürr, 2017).

Collagen is a crucial organic protein in skin and bone. Type I and type III collagen are synthesized from procollagen, which is obtained from dermal fibroblasts. Increasing scientific evidence has shown that collagen hydrolysate from fish skin gelatin prevents wrinkle formation, melanogenesis, and skin dryness. We reported previously that collagen or collagen hydrolysate supplementation contributes to good skin health (Kang et al., 2018; Lee et al., 2019; Kang et al., 2021; Park et al., 2021; Kim et al., 2022). Previous studies have reported that fish collagen peptide could weaken intestinal inflammation and treat

Received 31 October 2022; Revised 21 November 2022; Accepted 21 November 2022; Published online 31 December 2022

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diet-induced obesity and associated disorders (Astre et al., 2018; Rahabi et al., 2022). In this study, we investigated whether fish collagen peptide with different compositions of amino acids and peptides from other collagen hydrolysates could inhibit UVB irradiation-induced skin dryness, wrinkle formation, melanogenesis, and oxidative stress in the skin using cell and animal models. We investigated the moisturizing-related ceramide and HA synthesis factors, wrinkle-related factors, and melanogenesis-related factors to understand the fundamental mechanisms and effects of Naticol® on UVB-induced photoaging.

MATERIALS AND METHODS

Fish collagen peptide (Naticol®) preparation and standardization

Fish collagen peptide (Naticol®, Weishardt International, Graulhet, France), a purified fish collagen originating from tilapia (*Oreochromis* genus) skin, was provided by Tricombio Co., Ltd. (Seoul, Korea). Fish gelatin was hydrolyzed with enzymes and filtered through cellulose filters. Pasteurization was performed at 100～120°C for 60 s. The gelatin was then powdered using a spray dryer (NIRO®, GEA, Dusseldorf, Germany) under the following operating conditions: feeder, 900 L/h; inlet temperature, 150～180°C; outlet temperature, 60～90°C; spray pressure, 150～200 bars. Naticol® composed of type Ⅰ and Ⅲ collagen, is water soluble and neutral in odor and taste. The level of glycine-alanine-valine-glycine-proline-alanine (470.52 g/mol) peptide in Naticol® was 1.68 mg/g, determined by high-performance liquid chromatography (Vanquish Flex, Thermo Fisher Scientific, Waltham, MA, USA) coupled with a triple quadrupole mass spectrometer (MS/MS; TSQ Quantis, Thermo Fisher Scientific).

Cell culture and treatments

HaCaT (human keratinocytes) cells were provided by Professor Hwang of the College of Life Sciences, Kyung Hee University. Hs27 (human fibroblasts) and B16F10 (melanoma) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Each cell line was cultured, and cells were exposed to UVB or treated with 3-isobutyl-1-methylxanthine (IBMX) according to the methods described previously (Park et al., 2021; Kim et al., 2022). Each cell line was treated with ascorbic acid (100 μg/mL), arbutin (100 μg/mL), and Naticol® (100, 200, or 400 μg/mL).

Measurement of HA, sphingomyelin, pro-inflammatory cytokines, intracellular melanin, glutathione (GSH), tyrosinase, nitric oxide (NO), and cyclic adenosine monophosphate (cAMP)

HaCaT cells and B16F10 cells were lysed, and the levels HA, sphingomyelin, pro-inflammatory cytokines, melanin, GSH, tyrosinase, NO, and cAMP were measured according to the methods described previously (Park et al., 2021; Kim et al., 2022).

Animals

Forty-eight 5-week-old male SKH-1 hairless mice were obtained from SaeRon Bio (Uiwang, Korea) and accommodated in cages under automatically managed conditions (50±10% relative humidity, 12:12 h light/dark cycle, 22±2°C) during the experimental period. Mice were allocated into six groups (n=8 per group): normal control (NC; no UVB irradiation), control (C; irradiated with UVB), positive control 1 (PC 1; irradiated with UVB and administered L ascorbic acid at 200 mg/kg), positive control 2 (PC 2; irradiated with UVB and administered arbutin at 200 mg/kg), Naticol® 150 (irradiated with UVB and administered Naticol® 150 mg/kg), Naticol® 300 (irradiated with UVB and administered Naticol® 300 mg/kg). The UVB dose schedule was described previously (Park et al., 2021; Kim et al., 2022). The experiments were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (protocol no. KHGASP-KHGASP-21-576).

Measurement of skin hydration and histological observations

The hydration, wrinkle formation, and thickness of the dorsal skin were measured according to methods described previously (Park et al., 2021; Kim et al., 2022).

Measurement of antioxidant enzyme activity in the dorsal skin

The activities of superoxide dismutase (SOD), catalase, and GSH peroxidase (GPx) in the dorsal skin were measured according to methods described previously (Park et al., 2021; Kim et al., 2022).

Protein extraction and Western blot analysis

Protein isolation from cells and dorsal skin tissue and Western blot analysis were performed according to methods described previously (Park et al., 2021; Kim et al., 2022). The primary and secondary antibodies used for Western blot analysis are described in Table 1.

Isolation of total RNA and real-time polymerase chain reaction (PCR)

Total RNA isolation from cells and dorsal skin tissues and real-time PCR analysis were conducted according to methods described previously (Park et al., 2021; Kim et al., 2022). The primer pairs used for PCR are described in Table 2.

Table 1. Antibodies used for Western blot analysis

Biomarker	Distributor
CerS4 (LASS4)	Abcam (Cambridge, UK)
p65	Abcam
p-p65	Abcam
COX-2	Cell signaling (Beverly, MA, USA)
JNK	Cell signaling
p-JNK	Cell signaling
c-Fos	Cell signaling
p-c-Fos	Cell signaling
c-Jun	Cell signaling
p-c-Jun	Cell signaling
MMP-1	Abcam
MMP-3	Abcam
MMP-9	Abcam
Smad3	Cell signaling
p-Smad3	Cell signaling
PKA	Cell signaling
p-PKA	Cell signaling
CREB	Cell signaling
p-CREB	Cell signaling
MITF	Cell signaling
TRP-1	Abcam
TRP-2	Abcam
β-Actin	LSbio (Settle, WA, USA)

Host animal is rabbit.
Dilution for Western blot is 1:1,000.
CerS4, ceramide synthase 4; COX-2, cyclooxygenase-2; JNK, c-Jun N-terminal kinase; MMP, matrix metallopeptidase; PKA, protein kinase A; CREB, cAMP response element-binding protein; MITF, microphthalmia-associated transcription factor; TRP, tyrosinase-related protein.

Statistical analysis

All results were shown as mean±standard deviation. The data were statistically assessed using Duncan's multiple range test after one-way analysis of variance using SPSS software (SPSS Statistics v. 23.0, IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $P<0.05$.

RESULTS

Effects of Naticol® on factors related to skin moisturization in HaCaT cells exposed to UVB

To investigate the effect of Naticol® on the HA, sphingomyelin, and pro-inflammatory cytokines levels, we analyzed HaCaT cells exposed to UVB using ELISA. The levels of HA and sphingomyelin were significantly decreased in the control group compared to the NC group ($P<0.05$). The HaCaT cells exposed to UVB and treated with L-ascorbic acid or Naticol® showed a significant increase in HA and sphingomyelin levels compared to the control group ($P<0.05$) (Fig. 1A and 1B). The levels of the pro-inflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), showed a sig-

Table 2. Primer sets used for real-time polymerase chain reaction

Gene	Sequence (5′→3′)
HAS1 (M)	
Forward	TCA GGG AGT GGG ATT GTA GGA
Reverse	AAA TAG CAA CAG GGA GAA AAT GGA
HAS2 (M)	
Forward	AAT ACA CGG CTC GGT CCA AGT
Reverse	CCA TCG GGT CTG CTG GTT
HAS3 (M)	
Forward	GGC CAT GGG AGC TAA AGT TG
Reverse	CCA AAT TGA TGT TGA AAC TCT TGA AA
LCB1(SPT) (M)	
Forward	AGC GCC TGG CAA AGT TTA TG
Reverse	GTG GAG AAG CCG TAC GTG TAA AT
DEGS1 (M)	
Forward	CCG GCG CAA GGA GAT CT
Reverse	TGT GGT CAG GTT TCA TCA AGG A
Fibrillin-1 (M)	
Forward	ACA ATT GTT CAC CGA GTC GAT CT
Reverse	ACT GTA CCT GGG TGT TGC CAT T
TGF-β RI (M)	
Forward	CAT CCT GAT GGC AAG AGC TAC A
Reverse	TAG TGG ATG CGG ACG TAA CCA
Procollagen type I (M)	
Forward	TTA CGT GGC AAG TGA GGG TTT
Reverse	TGT CCA GAT GCA CTT CTT GTT TG
Collagen type I (M)	
Forward	GAC CGT TCT ATT CCT CAG TGC AA
Reverse	CCC GGT GAC ACA CAA AGA CA
GAPDH (M)	
Forward	CAT GGC CTT CCG TGT TCC TA
Reverse	GCG GCA CGT CAG ATC CA
HAS2 (H)	
Forward	GAA ACA GCC CCA GCC AAA
Reverse	AAG ACT CAG CAG AAC CCA GGA A
LCB1(SPT) (H)	
Forward	CCA TGG AGT GGC CTG AAA GA
Reverse	CTG ACA CCA TTT GGT AAC AAT CCT A
DEGS1 (H)	
Forward	GCT GAT GGC GTC GAT GTA GA
Reverse	TGA AAG CGG TAC AGA AGA ACC A
Elastin (H)	
Forward	GTC GGA GTC GGA GGT ATC
Reverse	TGA GAA GAG CAA ACT GGG
TGF-β R1 (H)	
Forward	TCC CGG CAG ATC AAC GA
Reverse	ACG CGG TCA CAA ACA TGG T
Procollagen (H)	
Forward	TCT CCT CCG AAG GGA ATG AAC
Reverse	CAG CGG TGA CAC TGA GAT CTG
Collagen type I (H)	
Forward	GCC TCG GAG GAA ACT TTG C
Reverse	TCC GGT TGA TTT CTC ATC ATA GC
GAPDH (H)	
Forward	CCC CAC ACA CAT GCA CTT ACC
Reverse	TTG CCA AGT TGC CTG TCC TT

M, mouse; H, human; HAS, hyaluronic acid synthase; LCB1(SPT), long chain base biosynthesis protein 1 (serine palmitoyltransferase); DEGS1, delta 4-desaturase sphingolipid 1; TGF-β RI, transforming growth factor beta receptor 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

nificant increase in the control group compared to the NC group ($P<0.05$). The HaCaT cells exposed to UVB and treated with L-ascorbic acid or Naticol® showed a significant decrease in the levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) compared to the control group ($P<0.05$). In particular, the levels of IL-1β and IL-6 in the Naticol 200 and 400 groups were not significantly different to those in the PC group ($P<0.05$) (Fig. 1C~1E).

To investigate the skin moisturizing-related factors of Naticol®, we analyzed HaCaT cells exposed to UVB using real-time PCR. The HaCaT cells exposed to UVB and treated with L-ascorbic acid and Naticol® showed significantly increased HA and ceramide synthesis-related factors, including mRNA expression of HA synthase (HAS) 2, ceramide synthase 4 (CerS4), delta 4-desaturase sphingolipid 1 (DEGS1), and elastin, compared with the control group ($P<0.05$) (Fig. 1F~1I). These results suggested that Naticol® collagen directly stimulated moisturizing factors in keratinocytes.

Effects of Naticol® on factors related to wrinkle formation in Hs27 cells exposed to UVB

To investigate the wrinkle-related factors of Naticol®, we analyzed Hs27 cells exposed to UVB using real-time PCR and Western blotting. The mRNA expression of transforming growth factor beta receptor 1 (TGF-β RI), procollagen type I, and collagen type I were significantly decreased in the control group compared to the NC group ($P<0.05$). The Hs27 cells exposed to UVB and treated with L-ascorbic acid or Naticol® showed significantly increased mRNA expression of TGF-β RI, procollagen type

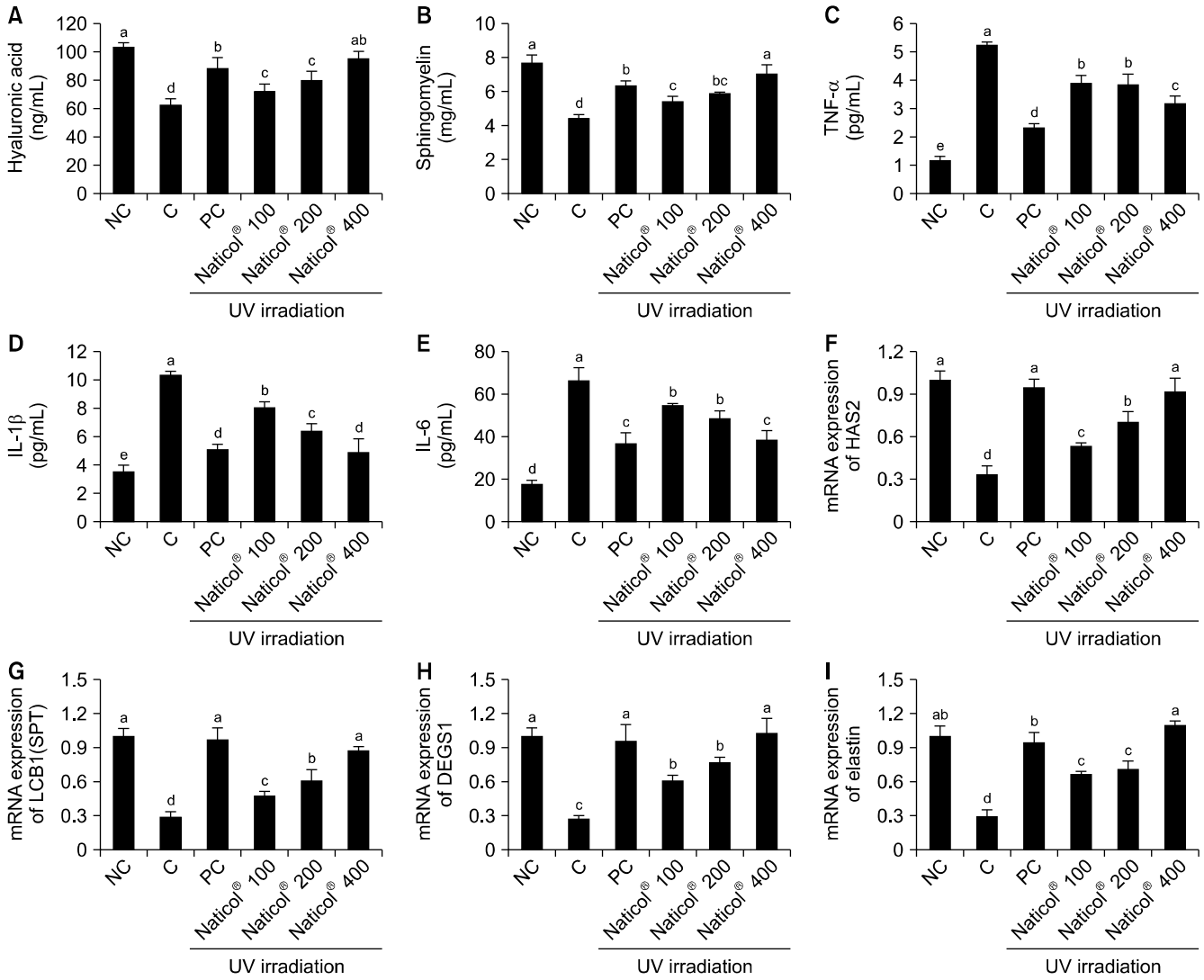

Fig. 1. Effects of Naticol® on skin moisture-related factors, hyaluronic acid (A), sphingomyelin (B), TNF-α (C), IL-1β (D), IL-6 (E), HAS2 (F), LCB1(SPT) (G), DEGS1 (H), and elastin (I), in HaCaT cells exposed to UVB. Cells were treated with UVB (50 mJ/cm^2), except NC, and incubated for 24 h with 100 μg/mL of L-ascorbic acid (PC) and various concentrations (100, 200, and 400 μg/mL) of Naticol® collagen. Values are presented as mean±SD. Different letters (a-e) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. Primers used for gene expression analysis are listed in Table 2. TNF, tumor necrosis factor; NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

I, and collagen type I compared to the control group ($P<0.05$) (Fig. 2A~2C). Moreover, the protein expression of p-c-Jun N-terminal kinase (JNK), p-c-Fos, p-c-Jun, matrix metallopeptidase (MMP)-1, MMP-3, and MMP-9 were significantly increased in the control groups compared to the NC group ($P<0.05$). The Hs27 cells exposed to UVB and treated with L-ascorbic acid or Naticol® showed a significant decrease in the protein expression of p-JNK, p-c-FOS, p-c-Jun, and all MMPs ($P<0.05$) (Fig. 2E~2J). Smad3 phosphorylation was significantly de-

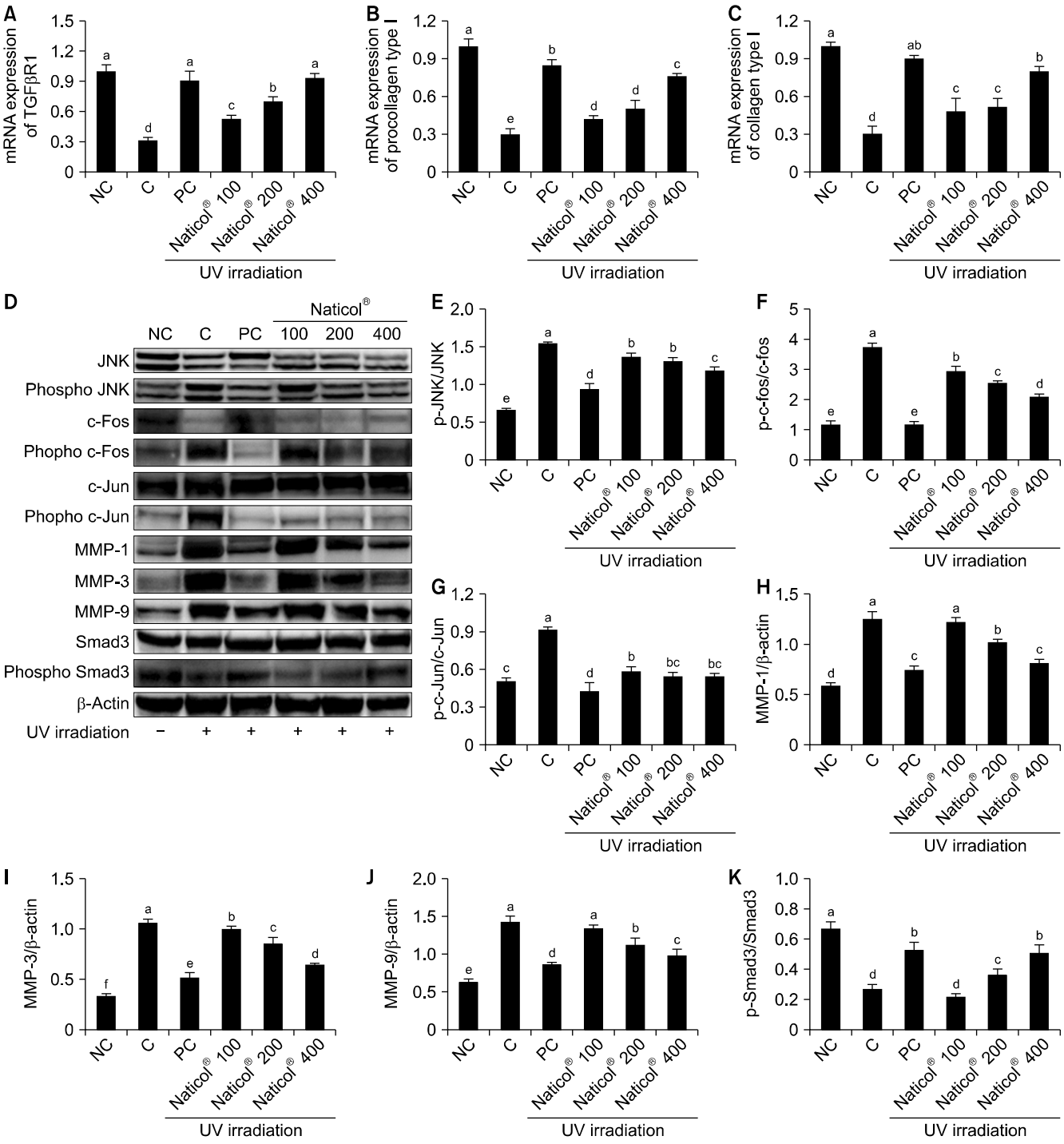

Fig. 2. Effects of Naticol® on skin wrinkle-related factors, TGF-β R1 (A), procollagen type I (B), collagen type I (C), protein band (D), p-JNK (E), p-c-FOS (F), p-c-Jun (G), MMP-1 (H), MMP-3 (I), MMP-9 (J), and p-Smad3 (K), in Hs27 cells exposed to UVB. Cells were treated with UVB (50 mJ/cm^2), except NC, and incubated for 24 h with 100 μg/mL of L-ascorbic acid (PC) and various concentrations (100, 200, and 400 μg/mL) of Naticol® collagen. Values are presented as mean±SD. Different letters (a-f) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. The biomarkers used for Western blot analysis are listed in Table 1. The primers used for gene expression analysis are listed in Table 2. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

creased in the control groups compared to the NC group ($P<0.05$). The Hs27 cells exposed to UVB and treated with L-ascorbic acid or Naticol® showed a significant increase in Smad3 phosphorylation ($P<0.05$) (Fig. 2K). These results indicated that Naticol® collagen directly controls wrinkle-related factors in keratinocytes.

Effects of Naticol® on factors related to melanogenesis in IBMX-treated B16F10 cells

To investigate the effects of Naticol® on melanin content, tyrosinase activity, melanogenesis-related factors, and the levels of NO, cAMP, and GSH, we analyzed IBMX-treated B16F10 cells using ELISA and Western blotting. The melanin content, tyrosinase activity, NO levels, and cAMP levels were significantly increased in the control; however, the levels of these factors were significantly decreased in the IBMX-treated B16F10 cells that received arbutin or Naticol® treatment compared with the control group ($P<0.05$) (Fig. 3A~3D). The GSH levels were significantly decreased in the control group compared with the NC group; however, IBMX-treated B16F10 cells that received arbutin or Naticol® treatment showed a significant increase in GSH level compared with the control group ($P<0.05$) (Fig. 3E). The protein expression of p-protein kinase A (PKA), p-cAMP response element-binding protein (CREB), microphthalmia-associated transcription factor (MITF), tyrosinase-related protein (TRP)-1, and TRP-2 was significantly increased in the control group compared with the NC group; however, IBMX-treated B16F10 cells that received arbutin or Naticol® treatment showed significant decreases in p-PKA, p-CREB, MITF, TRP-1, and TRP-2 expression compared with the control group ($P<0.05$) (Fig. 3F~3K). These results suggest that Naticol® collagen directly inhibited melanogenesis in melanocytes.

Effects of Naticol® on wrinkle formation, skin moisturization, and antioxidant activities in SKH-I hairless mice exposed to UVB

The changes in the morphology and histopathology of the dorsal skin of SKH-I hairless mice exposed to UVB are shown Fig. 4A. L-ascorbic acid, arbutin, or Naticol® consumption ameliorated the morphological and histopathological changes induced by UVB exposure, including wrinkle formation, epidermal thickness, and uneven skin. The skin moisturization was significantly decreased in the control group compared with the NC group; however, the L ascorbic acid, arbutin, and Naticol® supplementation groups showed significant increases in skin hydration ($P<0.05$) (Fig. 4B). Moreover, the antioxidant enzyme (SOD, catalase, and GPx) activities in the control group were significantly decreased compared with those in the NC group ($P<0.05$). The L-ascorbic acid, arbutin, and Naticol® supplementation groups showed significant increases in these activities compared to the control group ($P<0.05$). In particular, the Naticol® supplementation group showed a dose-dependent increase in skin hydration and SOD and catalase activities ($P<0.05$) (Fig. 4C~4E). These results indicate that Naticol® consumption effectively prevented UVB irradiation-induced morphological and histopathological changes and oxidative stress in dorsal skin.

Effects of Naticol® on skin moisturizing-related factors in SKH-I hairless mice exposed to UVB

To investigate the changes in skin moisturizing-related factors by Naticol® supplementation, we analyzed the dorsal skin from SKH-I hairless mice exposed to UVB using real-time PCR and Western blot analysis. The mRNA expression of HAS1~3, long chain base biosynthesis protein 1 of serine palmitoyltransferase [LCB1 (SPT)], delta 4-desaturase sphingolipid 1 (DEGS1), and fibrillin-1 in the control group was decreased compared with that in the NC group ($P<0.05$). The L-ascorbic acid, arbutin, and Naticol® consumption groups showed a significant increase in the mRNA expression of these factors compared to the control group ($P<0.05$). In particular, the Naticol® supplementation groups showed a dose-dependent increase in the mRNA expression of HAS1~3, LCB1(SPT), DEGS1, and fibrillin-1 ($P<0.05$) (Fig. 5A~5F). The protein expression of the ceramide synthesis-related factor, CerS4, in the control group was decreased compared with that in the NC group; however, the protein expression of HAS related factors, including p-IκBα, p-p65, and COX-2, in the control group was increased compared with that in the NC group ($P<0.05$). L-ascorbic acid, arbutin, or Naticol® supplementation conferred a significant increase in CerS4 protein expression, and significant decreases in the protein expression of IκBα, p65 phosphorylation, and COX-2 compared to the control group ($P<0.05$). In particular, the Naticol® supplementation groups showed a dose-dependent decrease in the protein expression of p-IκBα and p-p65 ($P<0.05$) (Fig. 5G~5K). These results indicate that Naticol® supplementation could effectively prevent UVB irradiation-induced skin dryness in dorsal skin.

Effects of Naticol® on factors related to wrinkle formation and melanogenesis in SKH-I hairless mice exposed to UVB

To investigate the changes in skin wrinkling and melanogenesis-related factors by Naticol® supplementation, we analyzed the dorsal skin from SKH-I hairless mice exposed to UVB using ELISA, real-time PCR, and Western blot analysis. The mRNA expressions of TGFβR1, procollagen type I, and collagen type I in the control group were decreased compared with the NC group ($P<0.05$). The L-ascorbic acid, arbutin, and Naticol® consumption groups showed a significant increase in the mRNA expressions

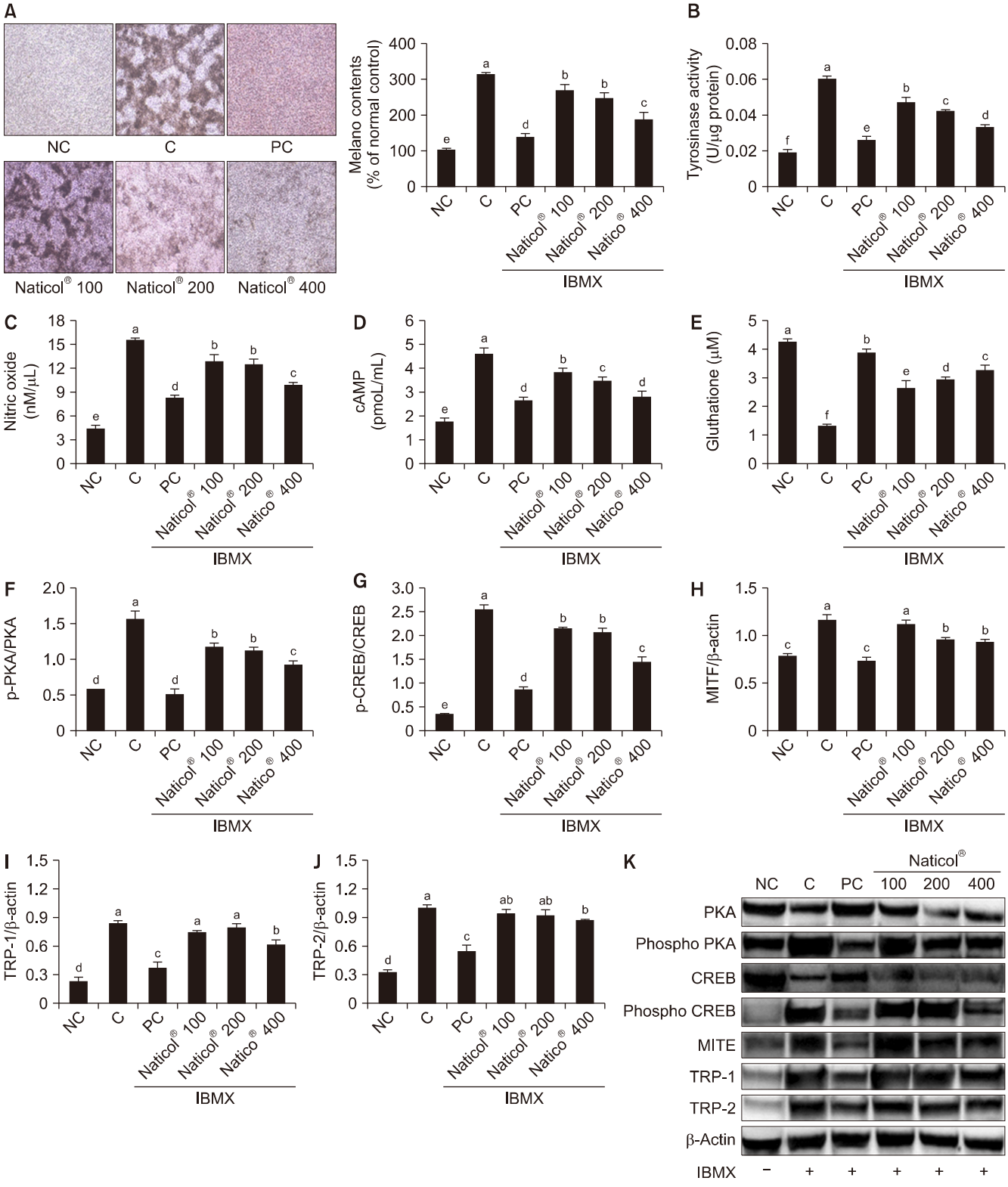

Fig. 3. Effects of Naticol® on skin melanogenesis-related factors, melanin contents (10×) (A), tyrosinase activity (B), nitric oxide (C), cAMP (D), glutathione (E), p-PKA (F), p-CREB (G), MITF (H), TRP-1 (I), and TRP-2 (J), and protein band (K) in IBMX-irradiated B16F10 cells. The cells were treated with 250 μM IBMX, except NC, and incubated for 24 h with 100 μg/mL of arbutin (PC) and various concentrations (100, 200, and 400 μg/mL) of Naticol® collagen. Values are presented as mean±SD. Different letters (a-f) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. The biomarkers used for Western blot analysis are listed in Table 1. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B; IBMX, 3-isobutyl-1-methyl-xanthine.

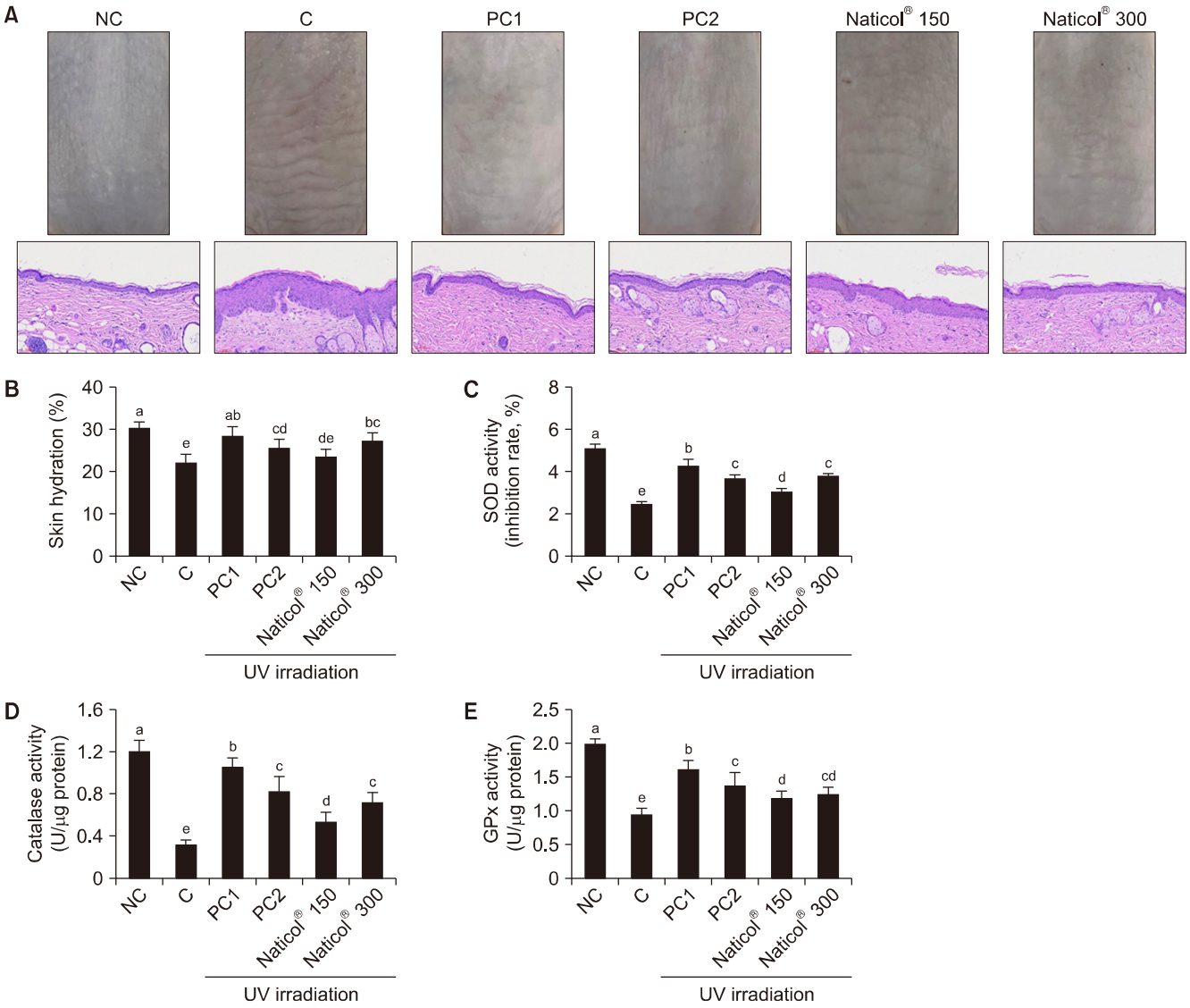

Fig. 4. Effects of Naticol® on morphological and histopathological changes (hematoxylin and eosin staining, 20×) (A), skin hydration (B), antioxidant activities of SOD (C), catalase (D), and GPx (E) in the dorsal skin of SKH-I hairless mice exposed to UVB. NC, AIN93G; C, UVB irradiation+AIN93G; PC1, UVB irradiation+AIN93G with L ascorbic acid (100 mg/kg); PC2, UVB irradiation+AIN93G with arbutin (100 mg/kg); Naticol®150, UVB irradiation+AIN93G with Naticol® collagen (150 mg/kg); Naticol®300, UVB irradiation+AIN93G with Naticol® collagen (300 mg/kg). Values are presented as mean±SD. Different letters (a-e) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

of these factors compared to the control group ($P<0.05$). In particular, the Naticol® consumption groups showed a dose-dependent increase in the mRNA expression of HAS1~3, LCB1(SPT), DEGS1, and fibrillin-1 ($P<0.05$) (Fig. 6A~6C). The expression of proteins involved in the JNK/c-Fos/c-Jun/MMPs pathway was significantly increased in the control group compared with the NC group; however, the L-ascorbic acid, arbutin, and Naticol® consumption groups showed significantly decreased protein expressions of p-JNK, p-c-Fos, p-c-Jun, MMP-1, MMP-3, and MMP-9 compared with the control group ($P<0.05$). In particular, the Naticol® consumption groups showed a dose dependent increase in the protein expressions of p-JNK, p-c-Fos, p-c-Jun, MMP-1, MMP-3, and MMP-9 compared to the control group ($P<0.05$) (Fig. 6D~6J). The control group showed a significant decrease in Smad3 phosphorylation compared to the NC group, and a significant increase was observed in Smad3 phosphorylation in the L-ascorbic acid, arbutin, and Naticol® consumption groups compared with the control group ($P<0.05$). In particular, the Naticol® consumption groups showed a dose-dependent increase in the protein expression of p-Smad3 compared to the control group ($P<0.05$) (Fig. 6K). The L-ascorbic acid, arbutin, and Naticol® consumption groups showed significant decreases in the levels of tyrosinase activity, NO, and cAMP, compared to control mice ($P<0.05$) (Fig. 7A~7C). In addition, the protein expression of p-PKA, p-CREB, MITF, TRP-1, and TRP-2 was decreased in the L ascorbic acid, arbutin, and Naticol® consumption groups compared to the control

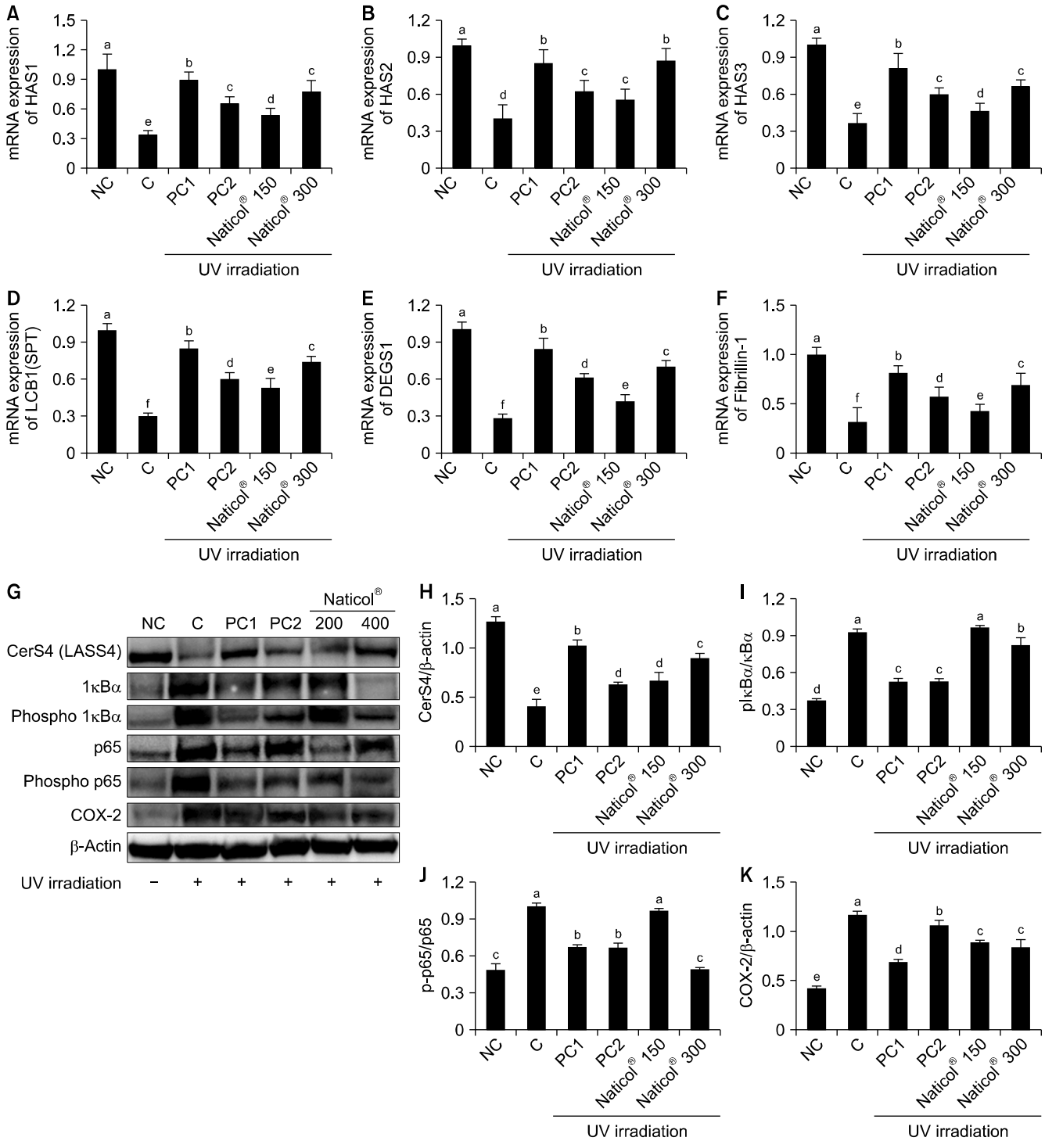

Fig. 5. Effects of Naticol® on skin moisture-related factors, HAS1 (A), HAS2 (B), LCB1(SPT) (D), DEGS1 (E), fibrillin-1 (F), protein band (G), CerS4 (H), p-IκBα (I), p-p65 (J), and COX-2 (K), in the dorsal skin of SKH-I hairless mice exposed to UVB. NC, AIN93G; C, UVB irradiation+AIN93G; PC1, UVB irradiation+AIN93G with L-ascorbic acid (100 mg/kg); PC2, UVB irradiation+AIN93G with arbutin (100 mg/kg); Naticol®150, UVB irradiation+AIN93G with Naticol® collagen (150 mg/kg); Naticol®300, UVB irradiation+AIN93G with Naticol® collagen (300 mg/kg). Values are presented as mean±SD. Different letters (a-f) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. Biomarkers used for Western blot analysis are listed in Table 1. Primers used for gene expression analysis are listed in Table 2. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

group ($P<0.05$). In particular, the Naticol® consumption groups showed dose-dependent decreases in tyrosinase activity, NO, and cAMP levels as well as a dose-dependent decrease in the protein expressions of p-PKA, p-CREB, MITF, TRP-1, and TRP-2 ($P<0.05$) (Fig. 7D~7I). These results indicate that Naticol® consumption could effectively attenuate UVB irradiation-induced skin wrinkling and melanogenesis in dorsal skin.

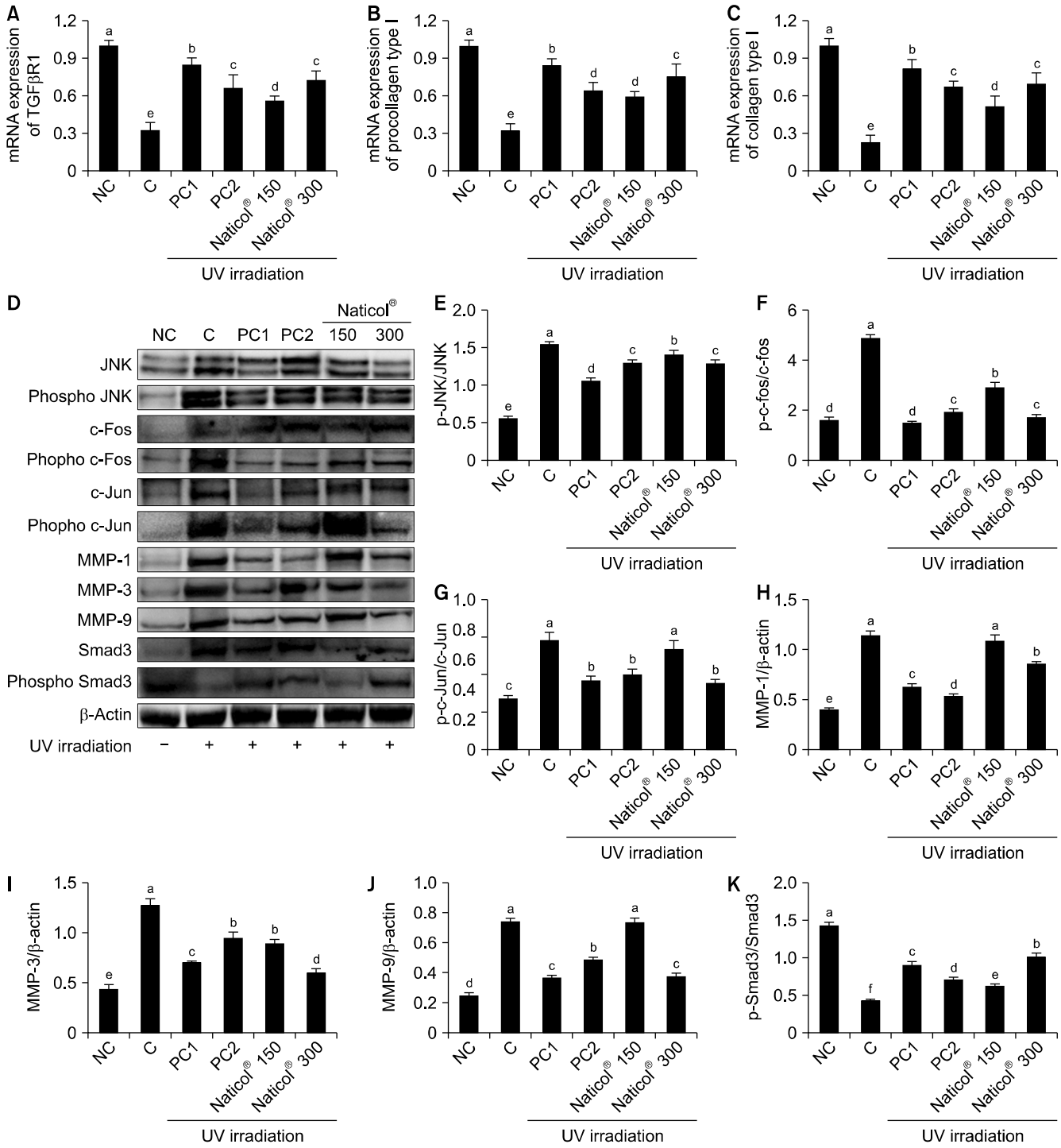

Fig. 6. Effects of Naticol® on skin wrinkle-related factors, TGFβR1 (A), procollagen type I (B), collagen type I (C), protein band (D), p-JNK (E), p-c-FOS (F), p-c-Jun (G), MMP-1 (H), MMP-3 (I), MMP-9 (J), and p-Smad3 (K), in the dorsal skin of SKH-I hairless mice exposed to UVB. NC, AIN93G; C, UVB irradiation+AIN93G; PC1, UVB irradiation+AIN93G with L-ascorbic acid (100 mg/kg); PC2, UVB irradiation+AIN93G with arbutin (100 mg/kg); Naticol®150, UVB irradiation+AIN93G with Naticol® collagen (150 mg/kg); Naticol®300, UVB irradiation+AIN93G with Naticol® collagen (300 mg/kg). Values are presented as mean±SD. Different letters (a-f) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. Biomarkers used for Western blot analysis are listed in Table 1. Primers used for gene expression analysis are listed in Table 2. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

DISCUSSION

Skin aging includes intrinsic factors, caused by inevitable physiological aging processes, and extrinsic factors caused by UV irradiation, temperature, and pollution. Consecutive exposure to UVB irradiation increases ROS production, which leads to the activation of inflammation, MMP production, and DNA damage. In addition, UV exposure

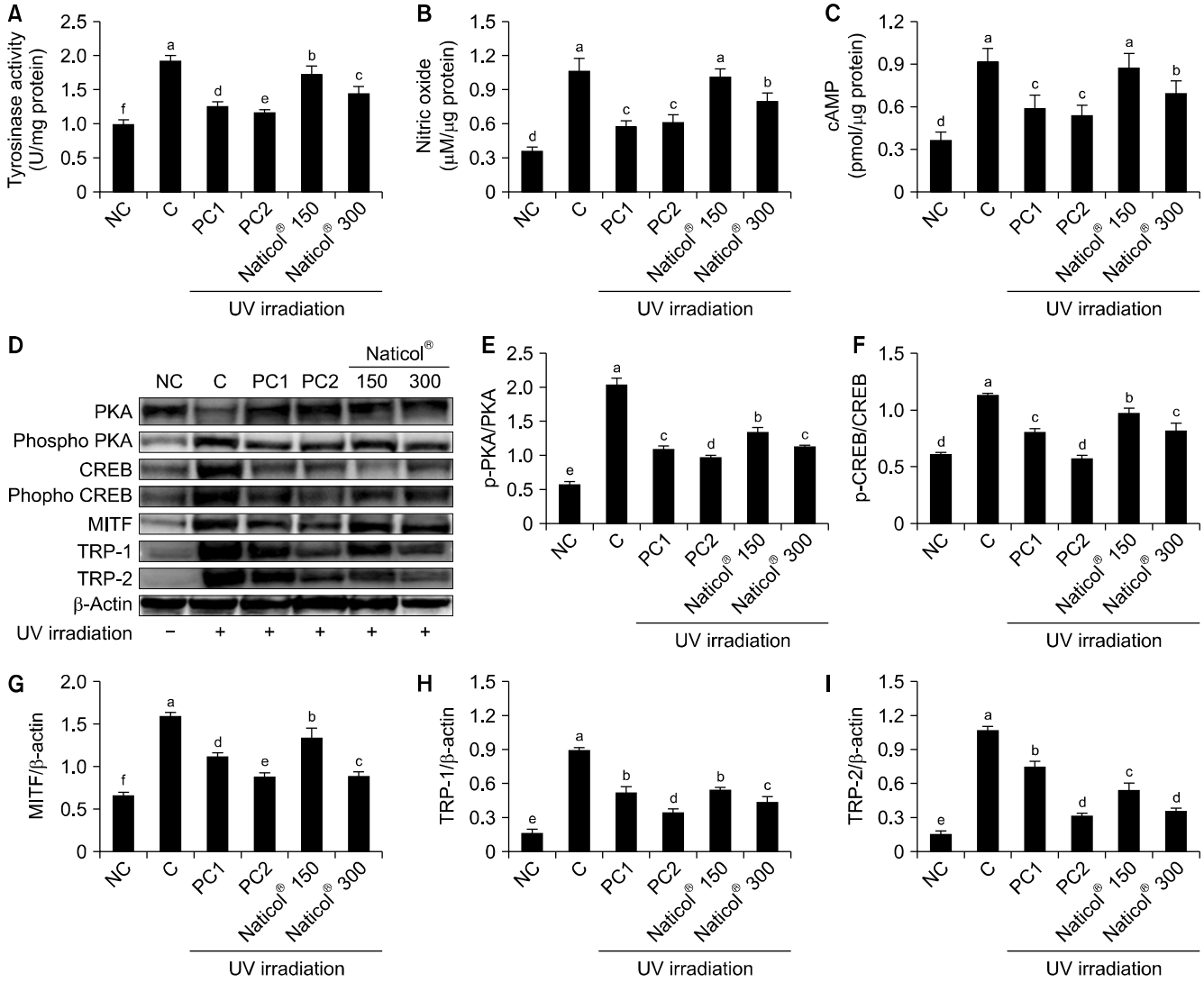

Fig. 7. Effects of Naticol® on skin melanogenesis-related factors, tyrosinase activity (A), nitric oxide (B), cAMP (C), protein band (D), p-PKA (E), p-CREB (F), MITF (G), TRP-1 (H), and TRP-2 (I) in the dorsal skin of SKH-I hairless mice exposed to UVB. NC, AIN93G; C, UVB irradiation+AIN93G; PC1, UVB irradiation+AIN93G with L-ascorbic acid (100 mg/kg); PC2, UVB irradiation+AIN93G with arbutin (100 mg/kg); Naticol®150, UVB irradiation+AIN93G with Naticol® collagen (150 mg/kg); Naticol®300, UVB irradiation+AIN93G with Naticol® collagen (300 mg/kg). Values are presented as mean±SD. Different letters (a-f) represent significant differences at $P<0.05$, as determined by Duncan's multiple range test. Biomarkers used for Western blot analysis are listed in Table 1. NC, normal control; C, control; PC, positive control; UVB, ultraviolet B.

triggers nuclear factor-kappa B pathway activation, which causes progressive inflammation and proteolysis via activation of the JNK pathway in the skin (Pillai et al., 2005; Chiang et al., 2013).

The present study investigated whether Naticol® could inhibit the skin damage caused by UVB-induced photoaging and oxidative stress via effects on skin hydration, wrinkle formation, and melanogenesis. Naticol® prevented wrinkle formation, loss of hydration, uneven skin, and increased antioxidant enzyme activities in SHK-I hairless mice that were exposed to UVB, suggesting that Naticol® suppresses skin photoaging caused by UVB irradiation.

Skin hydration is essential for preserving healthy skin and is necessary for skin barrier function. HA, a major element of the ECM, performs an important role in hydration balance due to its water-holding property. HA synthases are involved in HA synthesis at the inner plasma membrane. Previous studies have reported that UVB irradiation induces a loss of HA from the dermis and results in the inhibition of cell proliferation and migration (Wiest and Kerscher, 2008; Cavinato and Jansen-Dürr, 2017; Kobayashi et al., 2020). Ceramides also play an essential role in the water-holding property of the skin. The first step in the *de novo* synthesis of ceramides is catalyzation by serine palmitoyl transferase (SPT); SPT is then converted to sphingomyelins and glucosylceramides, which are then transported and secreted to connections in the stratum corneum and stratum granulosum (Coderch et al., 2003; Rabionet et al., 2014). In the present study, Naticol® increased HAS1~3, LCB1(SPT), and DEGS1 mRNA expression and CerS4 protein expression in both

cell and animal models exposed to UVB. These findings suggest that Naticol® controls hydration via the upregulation of HA and ceramide synthesis to ameliorate the loss in hydration caused by UVB irradiation.

Skin wrinkling is primarily related to the degradation of ECM protein via MMP secretion and collagen fragmentation. ROS and pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production caused by UVB irradiation result in excessive secretion of MMPs. MMPs participate in the degradation of different components of the ECM and membrane, including collagen. Collagen is the main structural protein of the ECM and is vital for connective tissue homeostasis. The TGF-β/Smad pathway regulates collagen synthesis. Previous studies have reported that UV irradiation upregulates the expression of MMPs via the MAPK pathway and downregulates the expression of collagen via the TGF-β/Smad pathway (Kondo, 2000; Jadoon et al., 2015; Lan et al., 2019; Ke and Wang, 2021). Our results showed that Naticol® promoted TGF-β/Smad3 pathway activation and inhibited JNK/MMP pathway activation and the production inflammatory cytokines in both cell and animal models exposed to UVB. These results suggest that Naticol® prevents wrinkle formation by activating collagen synthesis and suppressing MMP production.

Melanin, produced by melanocytes, plays a role in protecting the skin from UV irradiation. Melanogenesis is caused by inflammation or UV irradiation via adrenocorticotropic hormone or α-melanocyte-stimulating hormone. Tyrosinase controls the catalysis of L-tyrosine hydroxylation to levodopa. Tyrosinase expression is regulated by MITF, whose activation and expression are regulated by the cAMP/CREB pathway (Park et al., 2009; D'Mello et al., 2016). Our findings revealed that Naticol® protected against melanogenesis via GSH synthesis, tyrosinase activation, and inhibition of the cAMP/CREB/MITF pathway, in both cell and animal models exposed to UVB. Therefore, our results indicated that Naticol® ameliorated UVB irradiation-induced melanogenesis in melanocytes and SHK-I hairless mice.

We determined that Naticol® collagen prevented UVB-irradiated skin dryness, oxidative stress, skin wrinkling, and melanogenesis in the skin using cell and animal models. Naticol® collagen improved skin moisturization via HA and ceramide synthesis, and inhibited wrinkle formation and melanogenesis during UVB-irradiated oxidative stress. On the basis of these findings, we propose that the consumption of Naticol® collagen might be valuable for preventing skin photoaging.

FUNDING

None.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: ML, JL. Analysis and interpretation: ML, DK, SHP, JJ, WC, ARY. Data collection: ML, DK. Writing the article: ML, JL. Final approval of the article: all authors. Statistical analysis: ML. Overall responsibility: JL.

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