The Journal of Applied Pharmacology, **12**, 157-164 (2004)

Reduction of Photodamage by Topical Application of a Novel Anti-Wrinkle Agent Containing Growth Factors

Ju Mi KIM, Gook Jun AHN*, Young Sung SOHN, Kyung Koo KANG, Byoung Ok AHN, Jung Hwan LEE, Byung Moon KIM, Jong Won KWON and Won Bae KIM

Research Laboratories, Dong-A Pharm. Co. Ltd., 47-5, Sanggal, Kiheung, Yongin, Kyunggi 449-905, Korea

(Received September 1, 2004; Accepted September 15, 2004)

Abstract – DA-3711 is a novel anti-wrinkle agent containing growth factors derived from culture medium of artificial human skin. Photoprotective effect by DA-3711 against chronic UVB (ultraviolet B)-induced skin damage was investigated in hairless mice model. *Methods*: After hairless mice were irradiated to induce photodamage for 8 weeks with UVB, grouped mice were treated topically once a day with lotion base, DA-3711 (30% or 15%), Cylasphere retinol® (2500 I.U.), NouriCel® along with concomitant exposure to UVB for further 8 weeks. Then mice were sacrificed to assess photodamage-protective effect by replica analysis, biochemistry and histology. DA-3711 of 30% lotion significantly reduced UVB radiation-induced wrinkling, histological alterations and increased collagen contents. Whereas DA-3711 of 15% lotion and NouriCel® treatment showed a partial protective effect on skin wrinkle, epidermal and dermal thickness, and collagen content, Cylasphere retinol® showed no protective effects. These results demonstrate that topical application of DA-3711 can alleviate UVB-induced photodamage and potentially be used for reduction of UVB-induced photodamage.

Keywords □ DA-3711, growth factors, hairless mice, photodamage, ultraviolet B

INTRODUCTION

Chronic exposure of skin to ultraviolet B (UVB) radiation leads to the appearance of wrinkles and alterations in the composition of the skin. UVB irradiation causes severe damage to the underlying connective tissue, typically manifested in structural alterations in the epidermis and dermis. The major histopathologic change in skin exposed to UVB radiation is the accumulation of massive amounts of abnormal elastic tissue, termed solar elastosis (Bernstein *et al.*, 1994; Frances and Robert, 1984; Gilchrest, 1989; Mitchell, 1967; Montagna *et al.*, 1989; Taylor *et al.*, 1990; Warren *et al.*, 1991). Previous studies have reported that there is an accumulation of elastic fibers, a reduction and degeneration of collagen, and deposition of glycosaminoglycans (Oikarnin and Kallionen, 1989; Smith *et al.*, 1962), all of which lead ultimately to wrinkling and sagging.

There have been various attempts to protect the skin against UV-induced skin damage. UV-induced photodamaging could be decreased by antioxidants (Bissett *et al.*, 1990) and cytokines or growth factors such as transforming growth factor (TGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) (Circolo *et al.*, 1991; Edwards *et al.*, 1987; Kligman, 1987; Tiedemann *et al.*, 1997). Furthermore, it was reported that NouriCel® (Advanced Tissue Sciences Inc.), a topical agent containing growth factors derived from human tissue-engineered skin, ameliorates the facial wrinkles in clinical studies. The data suggest that such enriched solutions may be beneficial in the remodeling and regeneration of skin, thereby reducing the appearance of visible wrinkling. However, their results have not been published in peer-reviewed journals. It is pivotal to develop an optimal formulation to enhance skin-depth penetration of macromolecular growth factor without skin irritation.

DA-3711 is derived from the nutrient medium used to nourish three-dimensional human tissues in the manufacturing process of artificial skin (Dong-A pharmaceutical co. Ltd., Korea). It contains collagens and human growth factors such as vascular endothelial growth factor (VEGF), FGF and TGF-b1 produced by fibroblasts. The hairless mice have been used as an experimental model to investigate the effects of chronic UV irradiation. In this model, mice exposed to irradiation acquired

*Corresponding author
Tel: 82-31-280-1394, Fax: 82-31-282-8564
E-mail: ahnsnu2@donga.co.kr

fine wrinkles and sagging similar to those seen in humans with photodamage (Bissett *et al.*, 1987; Bryce *et al.*, 1998; Corcuff and Leveque, 1993; Kligman 1986). In the present study, the effect of DA-3711 on UVB-induced photodamage was investigated in hairless mice using replica analysis, histological examinations and biochemistry.

MATERIALS AND METHODS

Animals

Hairless (Skh: hr-1) female mice at 8 weeks of age were purchased from Japan SLC (Kotoh, Japan). Throughout the experiment, all animals were kept under standard laboratory conditions and allowed free access to food and UV-sterilized tap water. All experiments were performed in accordance with the institutional *standard procedure for animal care and experiments* (SOP-ANC) of the Dong-A Pharmaceutical Company and with the "Guide for the Care and Use of Laboratory Animals" from the National Institutes of Health.

Test materials

DA-3711 was obtained from the tissue engineering team of the Research Laboratories of Dong-A Pharm. Co. Ltd. (Kyunggi, Korea). It contains collagens and human growth factors such VEGF, FGF and TGF-b1 produced by neonatal cells. DA-3711 is formulated at either 30% or 15% on the basis of the lotion base, which consists of distilled water, caprylocaproyl macrogolglyceride (Labrasol®, west pharmaceutical services drug delivery & clinical research center, Nottingham, GB) isopropylmyristate, cetostearyl alcohol, propyleneglycol, glycerine, and tween 80. Cylasphere retinol® (Coletica, France) and NouriCel® (Biozhem Co. Ltd., USA) were purchased and used as controls for comparison. All other materials used in this study were obtained from Sigma Chemical Co..

Topical application

Mice were acclimatized for 1 wk before the experimental procedures began and were divided into six groups (group I-VI) of ten animals each. To group I, a non-irradiated, age-matched group of animals was included as a negative control. Groups II, III, IV, V and VI were exposed to UVB irradiation for 8 weeks. After an irradiation period of 8 weeks, animals were assigned to each treatment group. To groups II and III, Cylasphere retinol® (2500 I.U.) and NouriCel®, respetively, were applied topically to the dorsal skin in 100 ml aliquots immediately after UVB exposure. Groups IV, V and VI were assigned to DA-3711 treatment groups of 30%, 15% and 0% (lotion base), respectively. The dorsal skin of hairless mice was treated once daily for a further 8 weeks with concomitant UVB exposure.

UV Irradiation

The mice were irradiated using a bank of five UVB lamps (Philips), five times a week for 16 weeks. The lamps were approximately 30 cm distant from the mice. A progressive UVB exposure regimen was used starting at approximately 15 mJ/cm^2 (just below the erythemal dose) per exposure at week 1, and increasing by 10 mJ/cm^2 per week until week 4. The exposed dose at week 4 (45 mJ/cm^2) was then kept constant for the remaining period of exposure. The cumulative total dose received by the end of 16 weeks reached 3.3J/cm^2.

Grading scale for wrinkles

Wrinkling was graded blindly according to the previously performed method (Bryce *et al.*, 1987). Briefly, severity of wrinkling was graded 0 to 3: grade 0, no coarse wrinkles; grade 1, a few shallow coarse winkles; grade 2, some coarse wrinkles; grade 3, several deep coarse wrinkles. These assessments were performed weekly.

Skin replica analysis

The skin replica analysis, which is a quantitative method for the assessment of micro-topographic features, has been previously described (Kligman, 1986). Briefly, negative skin replicas were obtained with Provil® silicone (Heraeus Kulzer Inc.) from identical sites of the dorsal skin of hairless mice. A thin layer of freshly prepared Provil® silicone was gently spread over the bounded area of the ring and allowed to polymerize. This occurred within 5 min, and subsequently the ring was lifted from the skin together with the replica. Each specimen was coded and stored in an individual envelope. After slight anesthesia with ether, skin replicas were made before- and after-treatment of 4 weeks (week 12) or 8 weeks (week 16). The skin replicas were analyzed by means of the skin image processing system, EasyVision® (Professional Scientific Instrument Co., Ltd.). Briefly, the replicas were scanned and transferred into a computer and the image was digitized into 256×512 pixels with gray levels for brightness. The gray level values perpendicular to the main furrow axis of this digitized image were plotted. The resulting profiles could reflect the surface features at the selected location. Analysis of these plots yields three main parameters proportional to the degree of wrinkling and roughness of skin topography. The peak number is defined as the number of points of reflection within a line, and a higher num-

ber corresponds to the formation of more furrows. The length means the ratio of the entire length of the lineprofile in comparison to the length of the plane line. Ideally, the value of length is 100, indicating the absence of furrows. The height is defined as the average depth of furrow, which means the distance between the higher peak and the lower peak of furrows.

Histology and thickness of epidermis and dermis

After topical treatment for 4 and 8 weeks, five animals in each group were sacrificed. Strips of dorsal skin, 2 × 1 cm, were fixed in 10% buffered formalin, embedded in paraffin, and cut into sections which were stained with hematoxylin and eosin. For the epidermal and dermal thickness assessments (19), three slides per animal and three measurements per slide, a total of 9 measurements per animal, were made under X 100 magnification using a Zeiss (Axioskop2 plus) microscope.

Collagen measurements

Total collagen content in supernatants from skin homogenates was determined according to the producers technical information by using the Sircol collagen assay Kit® (Biocolor Ltd., USA). In brief, animals were sacrificed and the dorsal skin was washed thoroughly with saline. Next, 6 mm diameter skin-punch samples from the dorsal skin of 5 mice from each group of the 4- or 8-weeks treated animals were taken for examination. Individual skin samples were homogenized using sucrose buffer (0.32M sucrose, 0.01M Tris-HCl, 1mM EDTA, 5mM PMSF, pH 7.4). The homogenate was centrifuged at 12000 rpm for 10 min and the supernatant was collected for analysis. The supernatant was mixed with 2 ml of TBA-trichloroacetic acid-HCl solution (0.375% TBA/15% TCA in 0.25N HCl). The mixture was heated at 95 for 50 min and then cooled. After centrifugation at 12000 g for 10 min, the organic layer was removed and its absorbance at 540 nm was measured. For morphometric quantification, volumetric densities of collagen, that is the relative volume these structures occupy in tissue, were determined on paraffin sections using color analysis software. All analyses were performed with a Zeiss microscope connected to a video camera, which transferred the captured images to a microcomputer. Color based surface determinations on the digitized images were performed using a commercial software. Quantification, based on the area stained with Massons Trichrome stain, was performed at a final magnification of 400X. We analyzed three slides per animal and three fields per slide, a total of 9 fields per animal. Mean volumetric density value was determined for each animal.

Statistics

Values are expressed as mean±S.D. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for a multiple comparison. P values < 0.05 were considered significant.

RESULTS

Visual wrinkle changes

Throughout the experiment no significant irritation or inflammation was observed (data not shown). Moreover, the UVB induced neither erythema nor tumor. The degree of wrinkling is summarized in Fig. 1. As illustrated in the figure, 8 weeks of UVB exposure induced a significant increase in the wrinkling score. The skin progressively worsened over further 8-weeks of irradiation. The first significant difference in wrinkling score in comparison with the lotion base group was detected at 1 week and 3 weeks after application of 30% and 15% DA-3711 lotion, respectively (data not shown). Thereafter, a significant and dose-dependent reduction in the wrinkle score was observed persistently. Three weeks after treatment, NouriCel® treated animals also showed a significant reduction in wrinkle score. However, no restoration of wrinkles was observed in the Cylasphere retinol® treated-group.

Image analysis of skin replicas

Skin surface silicone replicas clearly showed the protective

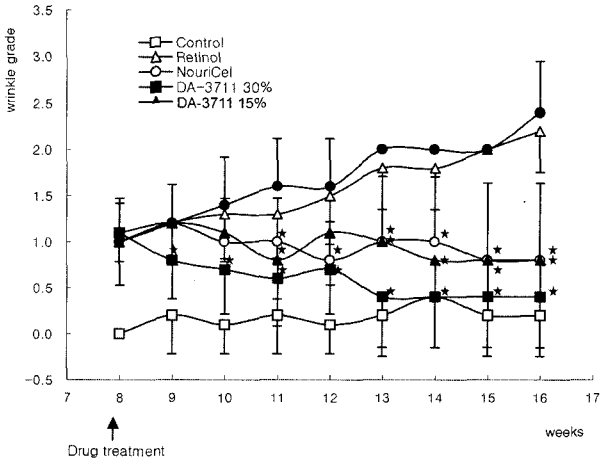

Fig. 1. The effects of DA-3711 on the change of wrinkle grade in UVB irradiated mice. DA-3711 15% (▲), 30% (■), Cylasphere retinol® (△), NouriCel® (○) or lotion base (●) were applied topically once a day for 8 weeks. Control group (□) comprised non-irradiated, age-matched animals. Data represent mean±S.D.; $P<0.05$.

effect of DA-3711 against wrinkle formation after 4 (data not shown) and 8 weeks of treatment (Fig. 2). When applied topically, DA-3711 showed a dose-dependent reduction in the wrinkle formation induced by chronic irradiation (Figs. 2E, F). The non-irradiated skin showed no wrinkle formation (Fig. 2A), whereas fixed, fine wrinkles were observed in lotion base- and Cylasphere retinol®- treated groups (Figs. 2B, C). NouriCel® treated animals showed an improvement in the wrinkle formation. However, the effects of reduction in wrinkle formation were marginal (Fig. 2D). Image analysis of replicas on skin surface parameters is presented in Fig. 3. None of the three parameters (peak number, length, height) were changed in the age-matched control group. However, in the lotion base group, chronic UVB irradiation induced an increase in peak number, length and height. DA-3711 showed the protective effect against the photodamages induced by chronic UVB irradiation. Thirty percent DA-3711 significantly decreased the peak number, length and height at both 4 and 8 weeks after treatment.

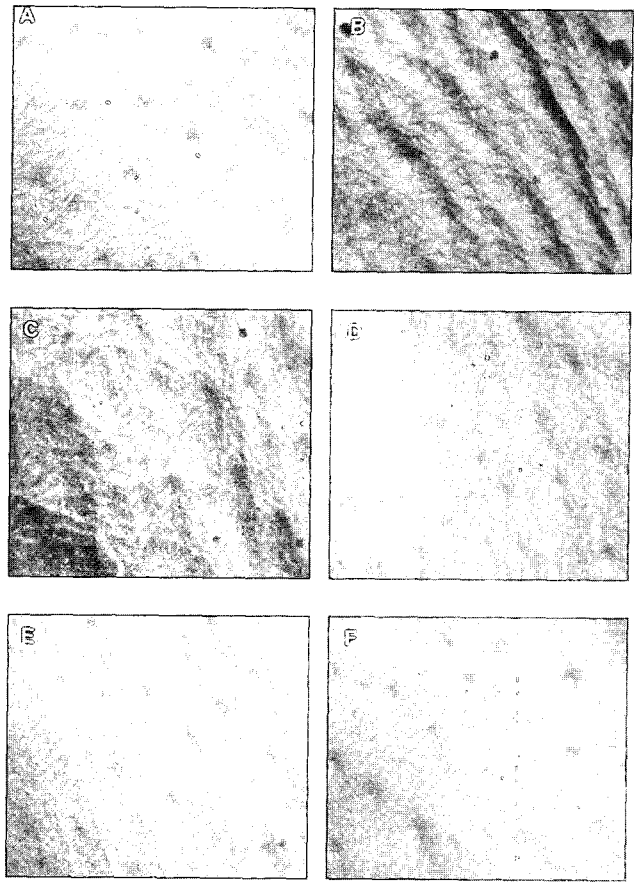

Fig. 2. Skin surface silicone replica taken from hairless mice after topical application of DA-3711 30% (F), 15% (E), NouriCel® (D), Cylasphere retinol® (C) or lotion base (B) for 8 weeks. Non-irradiated mice served as a normal control (A).

Both 15% DA-3711 and NouriCel® also showed the protective effect, but the potency was much less than that of 30% DA-3711. Cylasphere retinol® did not show the protective effect on the skin damage induced by chronic UVB irradiation.

Thickness of epidermis and dermis

Changes in epidermal and dermal thickness are shown in Table I. Chronic UVB irradiation significantly thickened the epidermis. The thickness of epidermis in age-matched control skin was 18.7 (±2.0)~23.6 (±2.2) mm, whereas that of irradiated mice at 12 weeks and 16 weeks was 44.1±2.3 mm and 55.3±2.1 mm, respectively. Topical application of DA-3711 on irradiated skin prevented epidermal thickening in a dose dependent manner. NouriCel® also reduced the UVB induced epidermal thickening. However, in the Cylasphere retinol® group, a significant epidermal thickening was observed. UVB irradiation alone also increased the dermal thickness significantly. The thickness of non-irradiated normal skin was 224.3(±17.3)~249.1(±12.8mm), and this value was increased to 479.6± 17.4 mm after 16 weeks of irradiation (Table I). Topical application of either DA-3711 or NouriCel® on irradiated skin prevented the dermal thickening. In contrast to DA-3711,

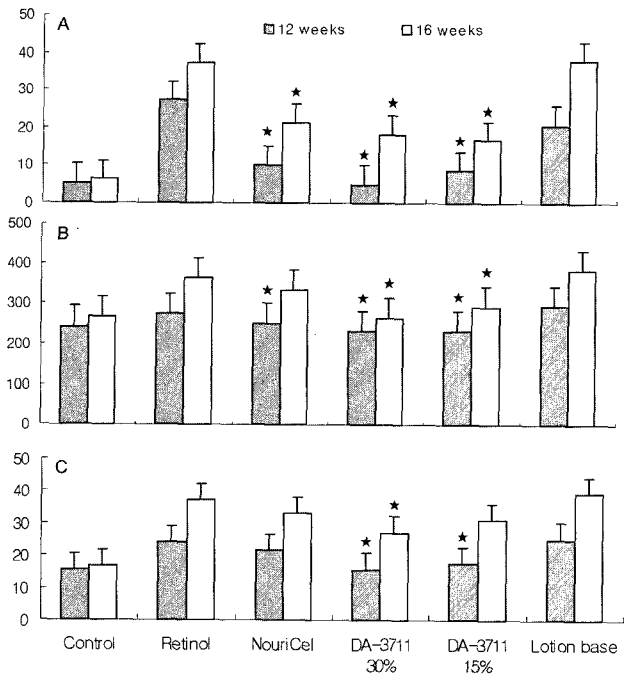

Fig. 3. Effects of DA-3711 on the skin surface parameters (peak number (A), length (B), height (C)). The parameters were produced by means of the EasyVision image processing system. Values represent mean±S.D. ★; Significantly different from the lotion base ($P<0.05$).

Table I. Effects of the topical application of DA-3711 on epidermal and dermal thickness

Groups	Treatment periods	Epidermis (μm)	Dermis (μm)
Control	4wk	18.7±2.0	224.3±17.3
	8wk	23.6±2.2	249.1±12.8
Cylasphere retinol®	4wk	47.9±3.5	386.3±25.1
	8wk	56.9±3.2	493.8±21.0
NouriCel®	4wk	25.9±1.9*	305.7±33.9*
	8wk	30.3±2.5*	344.5±26.0*
DA-3711 30%	4wk	20.9±2.0*	258.0±22.4*
	8wk	27.4±1.9*	313.3±18.8*
DA-3711 15%	4wk	26.0±2.1*	306.9±19.9*
	8wk	35.2±2.5*	382.2±17.2*
Lotion base	4wk	44.1±2.3	394.2±20.1
	8wk	55.3±2.1	479.6±17.4

Values represent mean±S.D. *; Significantly different from the lotion base ($P<0.05$).

Cylasphere retinol® treatment induced a significant increase in dermal thickness.

Histology

At week 12, the mice epidermis exposed to UVB irradiation was very thick and there were changes in several components of the dermis. Hyper-proliferation of all structures in the upper dermis, including fibroblasts and sebaceous cells, and the dermal cyst were observed. By week 16, the skin was clearly elastotic, with thick, tangled masses of elastic fibers in the dermis. The collagen, in the upper dermal area, was prominent and there were primarily increases in fibroblasts and mast cells. The entire dermis exhibited an increased in cellularity and slight infiltration of inflammatory cells (Fig. 4). In contrast to these animals, 4 or 8 weeks after topical application of 30% as well as 15% DA-3711 lotion markedly reduced these characteristic

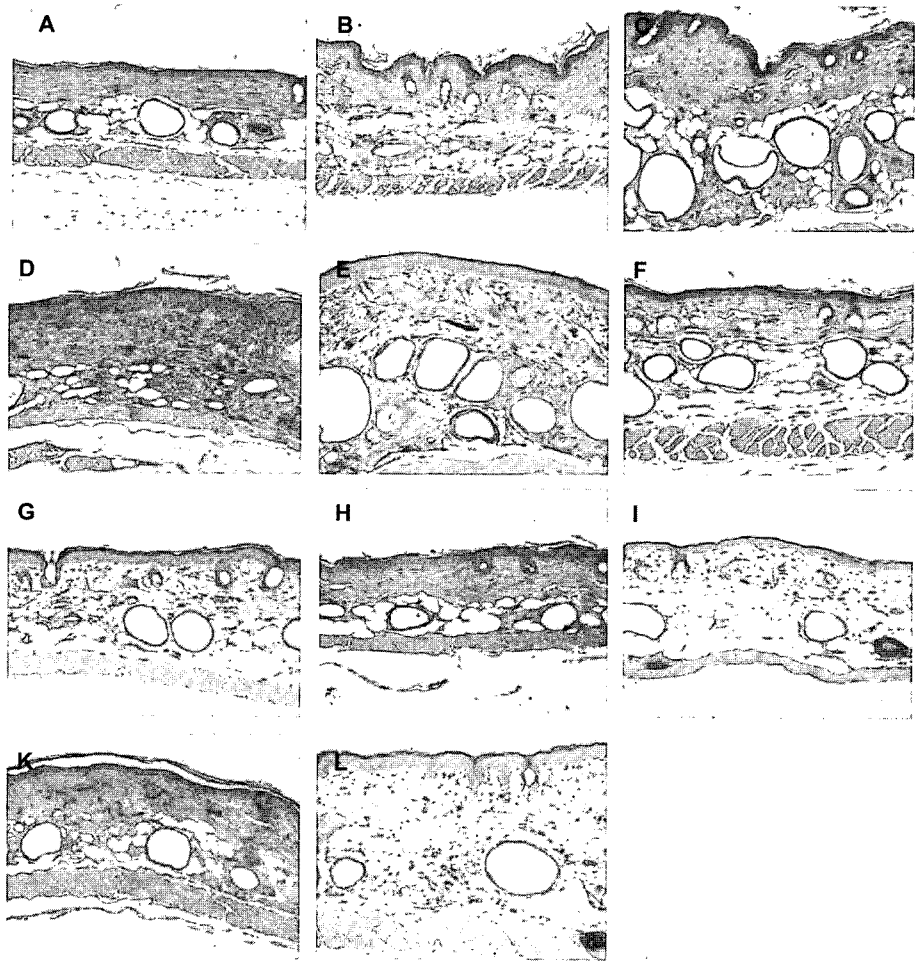

Fig. 4. Hairless mouse dorsal skin exposed to chronic UVB irradiation. Left (B, D, F, H) and right (C, E, G, I) columns were irradiated for 12 weeks and 16 weeks, respectively. A, Group I (age-matched control [16 weeks], no irradiation); B and C, Group II (lotion base, treated for 4 weeks (B) or 8 weeks (C)); D and E, Group III (Cylasphere retinol®); F and G, Group IV (NouriCel®); H and I, Group V (30% DA-3711 lotion); G and H, Group VI (15% DA-3711 lotion). H&E staining (X40).

alterations in dorsal skin exposed to chronic UVB irradiation. These protective effects peaked with DA-3711 lotion at the concentration of 30%. In the control groups, NouriCel® showed partial protective effects, but not the Cylasphere retinol® treated group (Fig. 4).

Collagen measurement

There were no statistical differences between the irradiated and non-irradiated groups in total collagen content in skin homogenates after 12 (data not shown) and 16 weeks (Fig. 5A) of UVB exposure. However, the collagen content was significantly increased in the groups treated with 30% and 15% DA-3711 lotion. The groups treated with Cylasphere retinol® or NouriCel® also showed an increase in collagen contents (Fig. 5A). In morphometric quantification using image analysis, volumetric densities of collagen were observed in the same patterns as described above. Chronic UVB irradiation did not induce a significant increase or decrease in the collagen content. However, DA-3711, Cylasphere retinol® and NouriCel® treatment increased the volumetric densities of collagen which were stained by Massons trichrome stain (Fig. 5B).

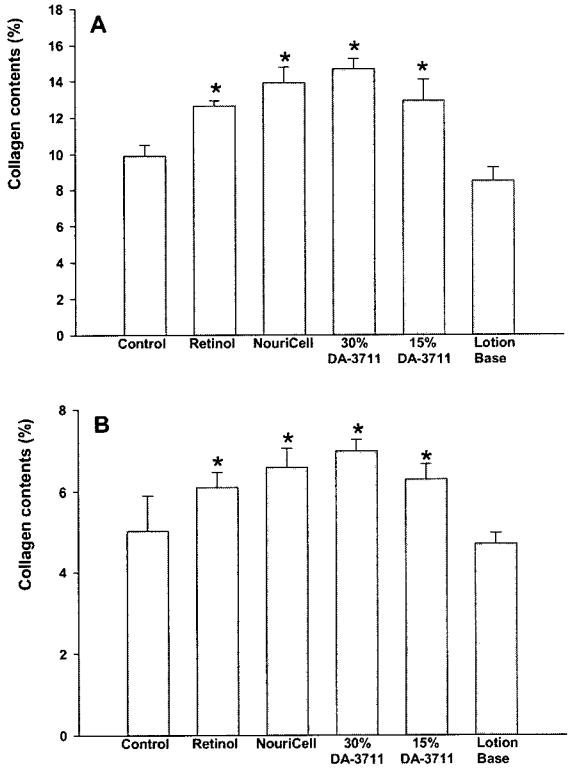

Fig. 5. The effect of DA-3711 on the collagen contents in skin homogenates (A) and morphometric analysis (B) after topical treatment for 8 weeks concomitant irradiation with UBV. Values represent mean±S.D. ★; Significantly different from the lotion base ($P<0.05$).

DISCUSSION

Chronic UV irradiation to the hairless mice dorsal skin has been reported to induce wrinkling, hyperplasia of the epidermis, conversion of the adipose tissue into fibrous tissue in the lower dermis, and finally an increase of chondroitin/dermatan sulfate content in the upper dermis (Mitani *et al.*, 2001). Some biochemical analyses of UV-exposed mouse skin have been reported (Chatterjee *et al.*, 1990; Kligman *et al.*, 1989). In addition, the histological and histochemical changes induced by chronic UV exposure in the skin of hairless mice mimicked those in actinically changed human skin (Koshiishi *et al.*, 1999). These results indicate that the hairless mice exposed to chronic UV irradiation are suitable model animals for examining photodamage.

Several materials such as antioxidant (Bissett *et al.*, 1990) growth factors (Circolo *et al.*, 1991; Edwards *et al.*, 1987; Kligman, 1987; Tiedemann *et al.*, 1997) retinoic acid (Olsen *et al.*, 1992; Weinstein *et al.*, 1991) and PARP inhibitor (Farkas *et al.*, 2002) have been tried to protect against the UV-induced skin damage. More recently, the Advanced Tissue Sciences Inc. developed an anti-wrinkle agent named NouriCel®, a topical solution containing growth factors derived from human tissue-engineered skin, and reported a reduction in facial wrinkles in studies of patients treated with it. DA-3711 is also a solution derived from culture that used to nourish three-dimensional human tissues in the manufacturing process developed by Dong-A pharmaceutical company for its line of human tissue-engineering products. It contains collagens and human growth factors such as VEGF, FGF and TGFb1 produced by neonatal cells. In this study, we studied the protective effect of DA-3711 against chronic UVB irradiation-induced skin injuries using a hairless mouse model, and demonstrated the protective effects of DA-3711 on the UVB-induced skin damage.

Until now, there have been few reports measuring the improvement of wrinkles objectively and quantitatively. This makes it difficult to evaluate the efficacy of anti-wrinkle agents. To resolve these shortcomings, we examined the efficacy of DA-3711 using computerized image analysis and compared changes to control groups. Based on analytic results of skin replica parameters (peak number, length and height), we concluded that topical application of DA-3711 is effective in the protection of skin roughness and wrinkling caused by UVB irradiation. Our data also demonstrated that histologically a

reduction occurred in the increased thickness of the epidermis and dermis of photodamaged skin after 4 and 8 weeks treatment. These results clearly demonstrated the protective effects of DA-3711 on UVB-induced skin damage in terms of wrinkle formation and histological alterations.

Although no histological correlation of wrinkling has been established, a reduction in collagen levels in areas of skin exposed to the sun is believed to be an etiologic component (Smith *et al.*, 1962). Wrinkle effacement in mice with photodamage was correlated with increased collagen synthesis (Chen *et al.*, 1992). In fact, when UV irradiated-mice are treated with topical retinoic acid, effacement of the wrinkles occurs in association with the appearance of a subdermal repair zone which is thought to represent the deposition of new collagen (Bryce *et al.*, 1988; Kligman *et al.*, 1984). Griffiths *et al.* (1993) postulated that collagen was responsible for the clinical improvement of wrinkles in mice. It was also assumed that a similar repair zone would prove to be the mechanism of wrinkle effacement in photodamaged human skin treated with retinoic acid. To investigate the underlying mechanism of DA-3711 on the antiwrinkle effect in photodamaged mice, we also investigated the effect of DA-3711 on the synthesis of collagen by measuring total collagen amount in the skin by biochemical and morphometric quantification methods. In our results, DA-3711 increased the amount of collagen content, significantly and dose-dependently, in both methods. These results indicate that reduced dermal collagen formation in photodamaged skin is partly prevented by treatment with DA-3711. Although the design of this study did not permit a direct correlation of collagen formation with the photoprotective effect of DA-3711, it is reasonable to presume that they may be correlated. Therefore, the ability of topical DA-3711 to reduce photodamage is not simply cosmetic but rather is probably based on preventing the decrease of dermal collagen and/or increasing the synthesis of total content of dermal collagen. Further studies are required to clarify how these changes occur, how they are related to wrinkle formation, and which type of collagen is changed. We are now undergoing a study to elucidate the exact mechanism by which DA-3711 protects against the photodamage induced by chronic UVB irradiation.

In conclusion, the protective effects of DA-3711 on photodamage were quantitatively assessed by computer assisted, image analysis using accurate and reproducible photographic techniques. Histological examinations and measurements of the thickness of the epidermis and dermis were also performed. In addition, in order to investigate the underlying mechanism of DA-3711, total collagen content in the skin was measured using biochemical and morphometric quantification methods. From these results, we concluded that topical application of DA-3711 dramatically protected the visible and histological skin changes induced by chronic UVB irradiation. Although the exact mechanism of this protection remains to be elucidated, the effect was related to the increase in collagen content, which suggests that DA-3711 may be a novel skin protective agent against UVB-induced photodamage.

REFERENCES

Bernstein, E.F., Chen, Y.Q. and Tamai K. (1994). Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. *J. Invest. Dermatol.* **103,** 182-186.

Bissett, D.L., Hannon, D.P. and Orr, T.V. (1987). An animal model of solar-aged skin: histological, physical, and visible changes in UV-irradiated hairless mouse skin. *Photochem. Photobiol.* **46,** 367-378.

Bissett, D.L., Chatterjee, R. and Hannon, D.P. (1990). Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol. Photoimmunol. Photome.* **7,** 56-62.

Bryce, G.F., Bogdan, N.J. and Brown, C.C. (1988). Retinoic acids promote the repair of the dermal damage and the effacement of wrinkles in the UVB-irradiated hairless mouse. *J. Invest. Dermatol.* **91,** 175-180.

Chatterjee, R., Benzinger, M.J., Ritter, J.L. and Bissett, D.L. (1990). Chronic ultraviolet B radiation-induced biochemical changes in the skin of hairless mice. *Photochem. Photobiol.* **51,** 91-97.

Chen, S., Kiss, I. and Tramposch, K.M. (1992). Effects of all-trans retinoic acid on UVB-irradiated hairless mouse skin. *J. Invest. Dermatol.* **98,** 248-254.

Circolo, A., Welgus, H.G., Pierce, G.F., Kramer, J. and Strunk, R.C. (1991). Differential regulation of the expression of proteinases/antiproteinases in fibroblasts; Effects of interleukin-1 and platelet-derived growth factor. *J. Biol. Chem.* **266,** 12283-12288.

Corcuff, P. and Leveque, J.L. (1993). Age related changes in skin microrelief measured by image analysis. In Aging skin. Edited by Leveque, J.L. and Agache, P.G. Marcel Dekker, New York, pp. 179-197.

Edwards, D.R., Murphy, G. and Reynolds, J.J. (1987). Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO. J.* **6,** 1899-1904.

Farkas, B., Magyarlaki, M., Csete, B., Nemeth, J., Rabloczky, G., Bernath, S., Literati Nagy, P. and Sumegi, B. (2002). Reduction of acute photodamage in skin by topical application of a novel PARP inhibitor. *Biochem. Pharmacol.* **63,** 921-932.

Frances, C. and Robert, L. (1984). Elastin and elastic fibers in normal and pathologic skin. *Int. J. Dermatol.* **23,** 166-179.

Gilchrest, B.A. (1989). Skin aging and photoaging: an overview. *J. Am. Acad. Dermatol.* **21,** 610-613.

Griffiths, C.E., Russman, A.N., Majmudar, G., Singer, R.S., Hamilton, T.A. and Voorhees JJ. (1993). Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *New. Engl. J. Med.* **329,** 530-535.

Kligman, L.H., Duo, C.H. and Kligman, A.M. (1984). Topical

retinoic acid enhances the repair of ultraviolet damaged dermal connective tissue. *Connect. Tissue. Res.* **12,** 139-150.

Kligman, L.H. (1986). Photoaging, manifestation, prevention, and treatment. *Dermatol. Clin.* **4,** 517-528.

Kligman, L.H. (1987). Full spectrum solar radiation as a cause of dermal photodamage; UVB to infrared. *Acta. Derm. Venereol. Stockholm.* **134,** 53-61.

Kligman, L.H., Gebre, M., Alper, R. and Kefalides, N.A. (1989). Collagen metabolism in ultraviolet irradiated hairless mouse skin and its correlation to histologic observations. *J. Invest. Dermatol.* **93,** 210-214.

Koshiishi, I., Horikoshi, E., Mitani, H. and Imanari, T. (1999). Quantitative alterations of hyaluronan and dermatan sulfate in the hairless mouse dorsal skin exposed to chronic UV irradiation. *Biochim. Biophys. Acta.* **1428,** 327-333.

Mitani, H., Koshiishi, I., Sumita, T. and Imanari, T. (2001). Prevention of the photodamage in the hairless mouse dorsal skin by kojic acid as an iron chelator. *Eur. J. Pharmacol.* **411,** 169-174.

Mitchell, R.E. (1967). Chronic solar dermatosis: a light and electron microscopic study of the dermis. *J. Invest. Dermatol.* **43,** 203-230.

Montagna, W., Kirchner, S. and Carlisle, K. (1989). Histology of sun-damaged skin. *J. Am. Acad. Dermatol.* **21,** 907-918.

Oikarnin, A. and Kallionen, M. (1989). A biochemical and immunohistochemical study of collagen in sun-exposed and protected skin. *Photodermatol.* **6,** 24-31.

Olsen, E.A., Katz, H.I., Levine, N., Shupack, J., Billys, M.M., Prawer, S., Gold, J., Stiller, M., Lufrano, L. and Thorne, E.G. (1992). Tretinoin emollient cream: a new therapy for photodamaged skin. *J. Am. Acad. Dermatol.* **26,** 215-224.

Smith, J.G. Jr., Davidson, E.A., Sams, W.M. Jr. and Clark, R.D. (1962). Alterations in human dermal connective tissue with age and chronic sun damage. *J. Invest. Dermatol.* **39,** 347-350.

Taylor, C.R., Stern, R.S., Leyden, J.J. and Gilchrest, B.A. (1990). Photoaging/photodamage and photoprotection. *J. Am. Acad. Dermatol.* **22,** 1-15.

Tiedemann, K., Malmstrom, A. and Westergren-Thorsson, G. (1997). Cytokine regulation of proteoglycan production in fibroblasts: separate and synergistic effects. *Matrix. Biol.* **15,** 469-478.

Warren, R., Gartstein, V., Kligman, A.M., Montagna, W., Allendorf, R.A. and Ridder, G.M. (1991). Age, sunlight, and facial skin: a histologic and quantitative study. *J. Am. Acad. Dermatol.* **25,** 751-760.

Weinstein, G.D., Nigra, T.P., Pochi, P.E., Savin, R.C., Allan, A., Benik, K., Jeffes, E., Lufrano, L. and Thorne, E.G. (1991). Topical tretinoin for treatment of photodamaged skin: a multicenter study. *Arch. Dermatol.* **127,** 659-665.