Tranexamic Acid Improves the Disrupted Formation of Collagen and Fibrillin-1 Fibers Produced by Fibroblasts Repetitively Irradiated with UVA

Karin Endo, Yoko Niki, Yukihiro Ohashi, and Hitoshi Masaki*

*Nippon Fine Chemical Co., Ltd.; 5–1–1 Umei, Takasago, Hyogo 676–0074, Japan; and bTokyo University of Technology; 1404–1 Katakura-machi, Hachioji, Tokyo 192–0982, Japan.

Received August 27, 2020; accepted November 24, 2020

The dermis is mainly constructed by type I collagen fibers, which provide mechanical strength to the skin by building a frame-like structure, and by elastic fibers, which provide elasticity to respond to movements of the skin. The depletion of collagen fibers and the disappearance of oxytalan fibers, which are a type of elastic fiber, are characteristic changes in photoaged skin. Prostaglandin E₂ (PGE₂) is one of the chemical mediators involved in inflammation and is responsible for sunburn. Furthermore, it has been reported that PGE₂ attenuates the production of collagen and the expression of elastic fiber-related factors in fibroblasts. Tranexamic acid (TXA), which is an anti-inflammatory medicine that inhibits plasmin, reduces the level of PGE₂ secreted following UV exposure or after inflammatory stimulation. However, few reports have verified TXA as an anti-skin aging agent. In this study, we examined the potential of TXA as an anti-skin aging agent using repetitively UVA-irradiated fibroblasts as a model for fibroblasts located in chronically sun-exposed dermis. Repetitively UVA-irradiated fibroblasts had higher secretion levels of PGE₂. In addition, fibroblasts repetitively irradiated with UVA or treated with PGE₂ produced disrupted collagen and fibrillin-1 fibers. Treatment with TXA improved the formation of both types of fibers by repetitively UVA-irradiated fibroblasts by restoring the expression of fiber-related proteins at the mRNA and protein levels. Thus, these results demonstrate that TXA has potential as an anti-photoaging agent.

Key words UVA; prostaglandin E₂; collagen; fibrillin-1; senescence-associated β-galactosidase; reactive oxygen species

INTRODUCTION

The causes of skin aging, which is characterized by wrinkles and sags, can be roughly classified into intrinsic dysfunctions with age and environmental influences, because the skin is located at the border between the inside of the body and the outer environment. Among environmental factors, the UV radiation in sunlight is a strong accelerator of skin aging, which is termed photoaging. UV is divided into UVA, UVB and UVC but little UVC reaches the earth’s surface. Considering the penetration into the skin, UVB reaches only to the basal layers of the epidermis, while UVA penetrates into the dermis. Photaged skin originates from structural alterations of the dermis. Thus, it is recognized that UVA is responsible for the progression of photoaging, because UVA directly affects dermal fibroblasts.

The dermis is mainly constructed of type I collagen fibers, which provide mechanical strength to the skin by building a frame-like structure, and elastic fibers, which provide elasticity to respond to movements of the skin. Although type I collagen fibers consist of relatively simple molecules, including collagen peptides 1A1 and 1A2, elastic fibers have a more complicated structure, i.e., they are tropoelastin-attached microfibrils. Microfibrils are formed by assembling fibrillin-1 and fibrillin-2 with the assistance of microfibrillar-associated protein 4 (MFAP4), elastin microfibril interface located protein 1 (EMILIN-1) and Latent transforming growth factor β (TGF-β) binding protein 4 (LTBP4). Tropoelastin is attached to microfibrils through coacervation with the assistance of fibulin-4 and fibulin-5 and is finally crosslinked by lysyl oxidase (LOX).

Structural alterations in photoaged skin include the depletion of collagen and the disappearance of oxytalan fibers, which are elastic fibers mainly composed of fibrillin-1 and fibrillin-2, in the papillary dermis, and increases of elastin fibers in the reticular dermis. It is recognized that those alterations are responsible for the appearance of wrinkles and sags in the skin.

Regarding the collagen depletion in photoaged skin, it has been demonstrated that reactive oxygen species (ROS) trigger the progression of photoaging. UV is a well-known generator of ROS, and promotes the decomposition of collagen fibers and suppresses their synthesis through the activation of c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) signaling triggered by ROS. Activated protein-1 (AP-1), which is regulated by JNK and NF-κB signaling, is a transcription factor controlling the expression of matrix metalloproteinase-1 (MMP-1), which is an enzyme that degrades Type I collagen, and of CCN1/Cyr61, which is a suppressor protein of collagen synthesis.

On the other hand, tranexamic acid (TXA), which is a well-known plasmin inhibitor, has anti-inflammatory effects due to the suppression of prostaglandin E₂ (PGE₂) synthesis, and in the dermatological field, TXA is widely used as a treatment for melasma. However, there are few reports about whether TXA is effective for preventing or improving photoaged skin. One recent study indicated...
the possibility that PGE$_2$ contributes to the progression of photoaging, because it suppresses collagen synthesis and the expression of elastic fiber-related proteins. In fact, UV and ROS also promote the synthesis of PGE$_2$ through increases of cyclooxygenase 2 (COX-2). Furthermore, it has been reported that senescent dermal fibroblasts also increase their release of PGE$_2$. Gathering these facts, it is expected that TXA has the potential to prevent or improve photoaged skin.

Thus, in this study we characterized the potential anti-skin aging effects of TXA using fibroblasts that were repetitively exposed to UVA as a model of senescent fibroblasts, focusing on the behavior of PGE$_2$.

MATERIALS AND METHODS

Materials TXA was purchased from Nippon Fine Chemical Co., Ltd. (Osaka, Japan). 5-Bromo-4-chloro-3-indolyl-$eta$-$d$-galactoside (X-gal) was purchased from Nacalai Tesque (Kyoto, Japan). Anti-collagen Type I was purchased from Rockland Immunochemicals (Pottstown, PA, U.S.A.). Streptavidin-horseradish peroxidase (HRP) was purchased from R&D Systems (Minneapolis, MN, U.S.A.). Fibrillin 1 monoclonal antibody was purchased from Invitrogen (Carlsbad, CA, U.S.A.). Goat anti-rabbit immunoglobulin G (H+L) secondary antibody, Alexa Fluor 546, were obtained from Abcam (Cambridge, U.K.). Streptavidin-horseradish peroxidase (HRP) was purchased from R&D Systems (Minneapolis, MN, U.S.A.). Alexa Fluor 546, and goat anti-mouse fluorescein diacetate (H$_2$DCFDA) was purchased from Merck (Darmstadt, Germany). The RNeasy Mini Kit was obtained from Qiagen (Hilden, Germany). ReverTra Ace qPCR RT Master Mix. qPCR was performed using the SYBR Green Gene Expression Assay with a Stepone Real-Time PCR System (Life Technologies, Carlsbad, CA, U.S.A.). GAPDH was purchased from TaKaRa BIO (Shiga, Japan).

Cell Culture Normal human dermal fibroblasts (NHDFs) were obtained from Kurabo Industries (Osaka, Japan), and were cultured in Dulbecco’s modified Eagle medium (DMEM) (Wako, Osaka, Japan) with 5% fetal bovine serum (FBS) (BIOWEST, Nuaillé, France) at 37°C in a 5% CO$_2$ atmosphere.

Repetitive UVA Irradiation of NHDFs NHDFs were seeded into multi-well plates in DMEM with 5% FBS, and after 24 h the cells were irradiated with UVA at a dose of 3 J/cm$^2$ once a day for 4 successive days. The UVA source used was a UV lamp (NEC, Tokyo, Japan) and irradiation energy was measured using a UVX radiometer (UVP, Upland, Canada). Sham-UVA-irradiated NHDFs were shielded from the UVA lamp during the UVA exposure. After the last UVA irradiation, NHDFs were cultured with or without TXA.

Senescence-Associated $eta$-Galactosidase (SA-$eta$-Gal) Activity SA-$eta$-gal activity, which is a marker of cell senescence, in NHDFs was evaluated using X-gal staining. NHDFs were fixed with 4% formaldehyde, and then were incubated with 1 mg/mL X-gal at 37°C for 24 h. After the treatment, the staining levels of X-gal in cells were observed using a microscope. SA-$eta$-gal activity in NHDFs was quantified by measuring the fluorescence intensity using a Cellular Senescence Assay kit (SA $eta$-Gal Activity) (Cell Biolabs, San Diego, CA, U.S.A.). Cells were recovered by trypsin treatment and the number of SA-$eta$-gal-positive cells per well was counted and is expressed as fluorescence intensity (F.I.) originating from SA-$eta$-gal-activity per number of cells.

Determination of Intracellular ROS Levels in NHDFs After repetitive irradiation of NHDFs with UVA (3 J/cm$^2$), the cells were incubated with or without TXA for 24 h and then were loaded with H$_2$DCFDA for 30 min. After lysing cells with phosphate buffered saline (PBS) containing 0.5% Triton X-100, the F.I. of each lysate was measured (Ex: 485 nm, Em: 530 nm). Protein concentrations in lysates were determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, CA, U.S.A.). Intracellular ROS levels are expressed as F.I. per µg protein.

Quantitative Real-Time PCR (qPCR) After repetitive irradiation with UVA (3 J/cm$^2$), NHDFs were incubated with or without TXA for 24 h. Total RNAs were purified from NHDFs using a RNeasy Mini Kit and were used for single-stranded cDNA synthesis by ReverTra Ace qPCR RT Master Mix. qPCR was performed using the SYBR Green Gene Expression Assay with a Stepone Real-Time PCR System (Life Technologies, Carlsbad, CA, U.S.A.). Data analysis was based on the ΔΔCt method with normalization by the housekeeping gene, GAPDH.

Quantification of PGE$_2$ Levels of PGE$_2$ in conditioned media (DMEM with 0.5% FBS) were quantified using an enzyme-linked immunosorbent assay (ELISA) (Enzo Life Science, Farmingdale, NY, U.S.A.). Protein concentrations in lysates were determined using a BCA protein assay kit.

Quantification of Type I Procollagen Procollagen levels in conditioned media (DMEM with 0.5% FBS) were quantified using a direct ELISA assay. Briefly, Type I collagen was used to prepare a calibration curve and conditioned media were incubated in 96-well ELISA plates (Sumitomo Bakelite, Tokyo, Japan) in aliquoted volumes for 2 h at room temperature in order to coat each sample on the bottom of each well. After blocking with 1% BSA (Sigma, Darmstadt, Germany) for 1 h, each well in the ELISA plate was incubated with 2 µg/mL rabbit anti-collagen Type I antibody at 4°C overnight, and then was further incubated with 40 ng/mL HISTOFINE simple stain MAX-PO (R) (Nichirei Corp., Tokyo, Japan) at room temperature for 1 h. After washing, levels of type I collagen were determined using the calibration curve by measuring the absorbance at 405 nm after incubation with 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (0.3 mg/mL) in 0.1M sodium citrate buffer (pH 4.0) using a microplate reader (Infinite F200, Tecan Group, Männedorf, Zürich, Switzerland). The number of cells was determined as the amount of protein in each cell lysate, which was dissolved in PBS containing 0.5% Triton X-100, using a BCA protein assay kit.

Immunostaining After repetitive irradiation with UVA (3 J/cm$^2$), NHDFs were incubated with or without TXA for 1 or 2 weeks, and were then fixed with 4% formaldehyde for 15 min. After blocking with 1% BSA (Sigma, Darmstadt, Germany), specimens were incubated with rabbit anti-collagen Type I polyclonal antibody (1:500) or mouse anti-fibrillin1 antibody (1:250) at 4°C overnight, followed by incubation with goat anti-rabbit IgG secondary antibody or goat anti-mouse IgG secondary antibody at room temperature for 2 h. Nuclei were stained with 4’6-diamidino-2-phenylindole (DAPI). Fluorescence images were observed using a fluorescence-
cence microscope (Keyence, Osaka, Japan). Fiber formations were analyzed in the captured images using image analysis software (BZ-H4M/measurement application, Keyence). In image analysis, the fluorescence intensity value was calculated by defining a fibrous structure of 100 \( \mu m^2 \) or more as a fiber.

**Statistical Analysis**
All data are expressed as means ± standard deviation (S.D.). Comparisons between two groups were performed using Tukey-test. A \( p \)-value of less than 0.05 is considered statistically significant.

**RESULTS**

**Intracellular ROS Levels, Senescence and Secretion of PGE\(_2\) in Repetitively UVA-Irradiated NHDFs**
NHDFs that were repetitively UVA-irradiated for 4 successive days had significantly higher levels of intracellular ROS and had significantly more SA-\( \beta \)-gal-positive cells compared with repetitively sham-UVA-irradiated NHDFs (Figs. 1a, d, e). As a result, repetitively UVA-irradiation significantly increased the secretion of PGE\(_2\) from NHDFs associated with the upregulation of COX2 (Fig. 2a). The mRNA expression level of COX2 in NHDFs treated with TXA (250 or 500 \( \mu M \)) for 24 h. (a) After loading with DCFDA for 30 min, the cells were rinsed and solubilized, and the fluorescence intensity (F.I.) of each lysate was measured (Ex, 485 nm; Em, 530 nm). (b) Total RNA was extracted from NHDFs and cDNAs were synthesized. The mRNA expression level of COX2 was measured by real-time PCR. (c) PGE\(_2\) levels in the conditioned media were quantified by ELISA. (d) Repetitively UVA-irradiated NHDFs were treated with or without TXA (500 \( \mu M \)) for 72 h, then were treated with 1 mg/mL X-gal, after which X-gal staining was observed using a microscope. (e) Senescence levels in repetitively UVA-irradiated NHDFs are expressed as fluorescence intensity F.I. originating from SA-\( \beta \)-gal-activity per number of cells. Scale bars: 50 \( \mu m \). Data are expressed as means ± S.D. from five independent experiments. Significance: * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \). (Color figure can be accessed in the online version.)

**Role of PGE\(_2\) Highly Secreted from Repetitively UVA-Irradiated NHDFs on the Formation of Collagen and Fibrillin-1 Fibers**
Since the culture of NHDFs in the presence of PGE\(_2\) for 1 or 2 weeks increased the population of SA-\( \beta \)-gal-positive cells (Fig. 4), it was expected that PGE\(_2\) also contributes to the formation of collagen and fibrillin-1 fibers. Indeed, treatment with PGE\(_2\) significantly decreased collagen secretion from NHDFs (Fig. 2b). These results suggested that repetitive UVA irradiation decreases the formation of both types of structural fibers by NHDFs. In fact, repetitively UVA-irradiated NHDFs showed poor formation of collagen and fibrillin-1 fibers (Figs. 3a–d).

**Effects of TXA on the Secretion of PGE\(_2\), SA-\( \beta \)-Gal Activity and Fiber Formation by Repetitively UVA-Irradiated NHDFs**
First, we examined whether TXA decreases the
secretion of PGE₂ from repetitively UVA-irradiated NHDFs, because TXA is known to suppress the secretion of PGE₂ due to its inhibition of plasmin. TXA elicited a significant decrease in the secretion level of PGE₂ from repetitively UVA-irradiated NHDFs and significantly downregulated COX2 mRNA expression levels (Figs. 1b, c). These results indicated that TXA has a substantial effect on NHDFs under these conditions. In addition, TXA also significantly reduced ROS levels in repetitively UVA-irradiated NHDFs, even though it is not an antioxidant (Fig. 1a). Further, TXA significantly suppressed the increased number of SA-β-gal-positive NHDFs that were induced by repetitive UVA irradiation (Figs. 1d, e).

Furthermore, treatment of repetitively UVA-irradiated NHDFs with TXA significantly ameliorated the decreases of type I collagen at both the mRNA and protein levels, and increased the MMP1 mRNA expression level (Fig. 2a). However, TXA failed to elicit a significant restoration of FBN1. On the other hand, treatment with TXA improved collagen and fibrillin-1 fibers, which showed a disrupted structure following repetitive UVA-irradiation (Fig. 3).

Effects of TXA on the PGE₂-Induced Decrease of Type I Collagen In order to elucidate the restoration mechanism of TXA in repetitively UVA-irradiated NHDFs, the effect of TXA on Type I collagen production suppressed by PGE₂ was examined. TXA did not show any restoration of Type I collagen production that was reduced by PGE₂ (Fig. 5b).
DISCUSSION

In general, skin aging, which is characterized by deep wrinkles and sags, appears as an end result of cumulative environmental stimuli. It is well recognized that the causes of skin aging are functional alterations that occur in dermal fibroblasts following the cumulative exposure to various stimuli, especially UVA, that remodel the dermal matrix. Thus, in order to determine the potential of various chemicals as anti-skin aging agents, it is essential to adopt a suitable experimental design, such as fibroblasts that are repetitively exposed to UVA.

Thus, we conducted this study to characterize the potential of TXA as an anti-photoaging agent using repetitively UVA-irradiated fibroblasts as a model. It has been reported that the characteristics of repetitively UVA-irradiated fibroblasts include higher levels of intracellular ROS and higher secretion levels of PGE₂. Thus, we first determined whether the characteristics of repetitively UVA-irradiated fibroblasts reproduce the phenomena observed in chronically sun-exposed elderly dermis focusing on the influence of PGE₂, because TXA is a typical anti-inflammatory drug that suppresses PGE₂ synthesis through the inhibition of plasmin. In fact, repetitively UVA-irradiated NHDFs show high levels of intracellular ROS and high secretion levels of PGE₂ (Figs. 1a, c). In addition, repetitive UVA-irradiation induced fibroblasts to a senescent phenotype showing a higher population of SA-β-gal-positive cells (Figs. 1d, e). In addition, repetitive UVA-irradiation induced a reduced function of the formation of collagen and fibrillin-1 fibers through the downregulation of COL1A1 and FBN1, and also enhanced the degradation of collagen fibers through the upregulation of MMP1 (Figs. 2, 3). In sun-exposed elderly dermis, the depletion of collagen fibers and the disappearance of oxtalan fibers that are mainly composed of fibrillin-1 and fibrillin-2 are typical alterations. Considering these results, the phenotype of repetitively UVA-irradiated fibroblasts is comparable to fibroblasts in sun-exposed elderly dermis. Then, we examined the contribution of PGE₂ to the phenotype of fibroblasts. NHDFs cultured in the presence of PGE₂ for a long time increased the population of SA-β-gal-positive cells (Figs. 4a, b). Regarding the mRNA expression levels of structural fiber-related proteins, treatment with PGE₂ accurately reproduced the phenotype of repetitively UVA-irradiated fibroblasts except for the mRNA expression level of FBN1 (Fig. 5a). Indeed, NHDFs cultured in the presence of PGE₂ showed that collagen and fibrillin-1 fibers had poor structures (Fig. 6). The sum of these results indicates that PGE₂ secreted from fibroblasts contributes to the structural...
alterations of the dermal matrix by influencing the synthesis of fiber-related proteins by fibroblasts in an autocrine fashion. However, despite the lack of a change in FBN1 mRNA levels in fibroblasts treated with PGE$_2$, fibrillin-1 fibers showed a poor structure. Since the reason for that is currently unclear, we will continue the study to clarify the action of PGE$_2$ on the formation of fibrillin-1 fibers.

Treatment with TXA improved the changes of repetitively UVA-irradiated NHDFs for the formation of collagen and fibrillin-1 fibers and suppressed the secretion level of PGE$_2$. TXA improved the formation of fibrillin-1 fibers, despite the failure to restore the FBN1 mRNA expression level which was downregulated by repetitive UVA irradiation. In the process of fibrillin-1 fiber formation, LTBP2 or LTBP4 are essential proteins. Although the potential impact of UVA on the status of LTBP2 and LTBP4 is unclear, it has been reported that LTBP2 is a substrate of plasmin. Thus, the inhibition of plasmin by TXA might be responsible for the improvement of fibrillin-1 fibers in repetitively UVA-irradiated fibroblasts.

Then, in order to rule out the direct action of TXA to restore collagen production, its effect on collagen levels following treatment with PGE$_2$ was examined (Fig. 5b). TXA did not show a restoration of collagen production reduced by PGE$_2$. On the other hand, it is presumed that plasmin levels increased in repetitively UVA-irradiated NHDFs because it has been reported that UVA increases urokinase-type plasminogen activator. Plasmin enhances PGE$_2$ synthesis through formation of a complex with annexin A2. Gathering these results, we consider that the mechanism underlying the effect of TXA originates from the suppression of PGE$_2$ synthesis through the inhibition of plasmin and through reductions of intracellular ROS. On the other hand, despite the complete restoration of PGE$_2$ elicited by TXA in repetitively UVA-irradiated NHDFs, the restoration of COL1A1 and MMP-1 by TXA did not reach the levels of sham-UVA irradiation. These facts suggest that altered mRNA expression levels may progress through another pathway other than PGE$_2$. ROS in NHDFs generated by repetitive UVA irradiation regulates mRNA expression levels of COL1A1 and MMP-1 through AP-1 dependent transcription. Because the reduction of intracellular ROS by TXA in repetitively UVA-irradiated NHDFs was not complete, the ROS that remained might be responsible for the altered mRNA expression of COL1A1 and MMP-1.

On the other hand, TXA reduced the levels of intracellular ROS in repetitively UVA-irradiated NHDFs despite the fact that TXA does not contain any functional group with antioxidation properties. At the moment, it is difficult to rule out that reducing intracellular ROS levels by TXA may be a basic function to achieve the improvement of fiber formation since it has been shown that the synthesis of PGE$_2$ is stimulated by ROS. However, TXA, which functions like an antioxidant even though it is not an antioxidant, is a new feature. Thus, we will continue further studies to elucidate this feature of TXA, and will report those findings elsewhere.

The results of this study demonstrate the potential of TXA to act as an anti-skin aging agent via changes in repetitively UVA-irradiated NHDFs used as a model of UVA-damaged fibroblasts located in sun-exposed elderly dermis, in the remodeling of collagen and fibrillin-1 fibers. Repetitively UVA-irradiated fibroblasts had a decreased formation of collagen and fibrillin-1 fibers, and treatment with TXA was able to restore those defects. Since most functional changes in repetitively UVA-irradiated fibroblasts were reproduced by PGE$_2$, we conclude that TXA prevents or improves those changes through the suppression of PGE$_2$ synthesis in repetitively UVA-irradiated fibroblasts and thus, TXA has the potential of an effective anti-skin aging agent.
REFERENCES

Conflict of Interest  Karin Endo, Yoko Niki and Yukihiro Ohashi are employees of Nippon Fine Chemical Co., Ltd.

Battie C, Verschoore M. Cutaneous solar ultraviolet exposure and clinical aspects of photo-damage. Indian J Dermatol. Venereol. Leprol., 78 (Suppl. 1), S9–S14 (2012).

Noda K, Dabovic B, Takagi K, Inoue T, Horiguchi M, Hirai M, Fujikawa Y, Akama TO, Kasumoto K, Zilberberg L, Sakai LY, Koji K, Naitoh M, von Melchner H, Suzuki S, Ritkon DB, Nakamura T. Latent TGF-β binding protein 4 promotes elastic fiber assembly by interacting with fibrilin-5. Proc. Natl. Acad. Sci. U.S.A., 110, 2852–2857 (2013).

Kadoya K, Sasaki T, Kostka G, Timpl R, Matsuzaki K, Kumagai N, Sakai LY, Nishiyama T, Amano S. Fibrillin-5 deposition in human skin: decrease with ageing and ultraviolet B exposure and increase in solar elastosis. Br. J. Dermatol., 153, 607–612 (2005).

Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. J Investig Dermatol. Symp. Proc., 14, 20–24 (2009).

Varani J, Schuger L, Dane MK, Leonard C, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ. Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photo-damaged skin. J. Invest. Dermatol., 122, 1471–1479 (2004).

Lim TG, Jung SK, Kim JE, Kim Y, Lee HJ, Jang TS, Lee KW. NADPH oxidase is a novel target of delphinidin for the inhibition of βT. Latent TGF-K, Naitoh M, von Melchner H, Suzuki S, Rifkin DB, Nakamura T. Latent TGF-β binding protein 4 promotes elastic fiber assembly by interacting with fibrilin-5. Proc. Natl. Acad. Sci. U.S.A., 110, 2852–2857 (2013).

Kadoya K, Sasaki T, Kostka G, Timpl R, Matsuzaki K, Kumagai N, Sakai LY, Nishiyama T, Amano S. Fibrillin-5 deposition in human skin: decrease with ageing and ultraviolet B exposure and increase in solar elastosis. Br. J. Dermatol., 153, 607–612 (2005).

Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. J Investig Dermatol. Symp. Proc., 14, 20–24 (2009).

Varani J, Schuger L, Dane MK, Leonard C, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ. Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photo-damaged skin. J. Invest. Dermatol., 122, 1471–1479 (2004).

Lim TG, Jung SK, Kim JE, Kim Y, Lee HJ, Jang TS, Lee KW. NADPH oxidase is a novel target of delphinidin for the inhibition of UVB-induced MMP-1 expression in human dermal fibroblasts. Exp. Dermatol., 22, 428–430 (2013).

Desai S, Ayres E, Bak H, Manco M, Lynch S, Raab S, Du A, Green DT, Skobowiat C, Wangari-Talbot J, Zheng Q. Effect of a tranexamic acid, kojic acid, and niacinamide containing serum on facial dermat. Exp. Dermatol., 22, 428–430 (2013).

Li D, Shi Y, Li M, Liu J, Feng X. Tramexamic acid can treat ultraviolet radiation-induced pigmentation in guinea pigs. Eur. J. Dermatol., 20, 289–292 (2010).

Li Y, Lei D, Swindell WR, Xia W, Weng S, Fu J, Worthen CA, Okubo T, Johnston A, Gadjojonsen JE, Voorhees JJ, Fisher GJ. Age-associated increase in skin fibroblast-derived prostaglandin E2 contributes to reduced collagen levels in elderly human skin. J. Invest. Dermatol., 135, 2181–2188 (2015).

Rundhaug JE, Fischer SM. Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. Photochem. Photobiol., 84, 322–329 (2008).

10) Rundhaug JE, Fischer SM. Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. Photochem. Photobiol., 84, 322–329 (2008).

11) Yokota M, Yahagi S, Tokudome Y, Masaki H. Chymyl alcohol suppresses PG2 synthesis by human epidermal keratinocytes through the activation of PPAR-γ. J. Oleo Sci., 67, 455–462 (2018).

12) Yoshimoto S, Yoshida M, Ando H, Ichihashi M. Establishment of photoaging in vitro by repetitive UVA irradiation: induction of characteristic markers of senescence and its prevention by PAPLAL with potent catalase activity. Photochem. Photobiol., 94, 438–444 (2018).

13) Hiramoto K, Yamate Y, Kobayashi H, Ishii M. Long-term ultraviolet A irradiation of the eye induces photoaging of the skin in mice. Arch. Dermatol. Res., 304, 39–45 (2012).

14) Chang WC, Shi GY, Chow YH, Chang LC, Hau JS, Lin MT, Jen CJ, Wing LY, Wu HL. Human plasmin induces a receptor-mediated arachidonate release coupled with G proteins in endothelial cells. Am. J. Physiol., 264, C271 (1993).

15) Maeda K, Naganuma M. Topical trans-4-aminomethylcyclohexane-carboxylic acid prevents ultraviolet radiation-induced pigmentation. J. Photochem. Photobiol. B, 87, 136–141 (1998).

16) Suwabe H, Serizawa A, Kajiwara H, Ohkido M, Tsutsuji Y. Degenerative processes of elastic fibers in sun-protected and sun-exposed skin: immunoelectron microscopic observation of elastin, fibrillin-1, amyloid P component, lysozyme and alpha1-antitrypsin. Pathol. Int., 49, 391–402 (1999).

17) Fujikawa Y, Yoshida H, Inoue T, Ohbayashi T, Noda K, von Melchner H, Iwasaka T, Shiojima I, Akama T, Nakamura T. Latent TGF-β binding protein 2 and 4 have essential overlapping functions in microfibril development. Sci. Rep., 7, 43714 (2017).

18) Hyytiainen M, Taipale J, Heldin CH, Keski-Oja J. Recombinant latent transforming growth factor b-binding protein 2 assemble to fibroblast extracellular matrix and is susceptible to proteolytic processing and release. J. Biol. Chem., 273, 20669–20676 (1998).

19) Rotem N, Axelrod JH, Miskin R. Induction of urokinase-type plasminogen activator stimulation of monocyte matrix metalloproteinase-1 production is mediated by a UV-induced secreted protein. Mol. Cell. Biol., 7, 622–631 (1987).

20) Zhang Y, Zhou ZH, Bugge TH, Wahl LM. Urokinase-type plasminogen activator stimulation of monocyte matrix metalloproteinase-1 production is mediated by plasmin-dependent signaling through annexin A2 and inhibited by inactive plasmin. J. Immunol., 179, 3297–3304 (2007).

21) Hwang YP, Oh KH, Yun HJ, Jeong HG. The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. J. Dermatol. Sci., 61, 23–31 (2011).