Modulation of Skin Collagen Metabolism in Aged and Photoaged Human Skin *In Vivo*

Jin Ho Chung, Jin Young Seo, Hai Ryung Choi, Mi Kyung Lee, Choon Shik Youn, Gi-eun Rhie, Kwang Hyun Cho, Kyu Han Kim, Kyung Chan Park, and Hee Chul Eun

Department of Dermatology, Seoul National University College of Medicine, Seoul, and Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea

To the best of our knowledge, no study has been conducted to date to directly compare the collagen metabolism of photoaged and naturally aged human skin. In this study, we compared collagen synthesis, matrix metalloproteinase-1 levels, and gelatinase activity of sun-exposed and sun-protected skin of both young and old subjects. Using northern blot analysis, immunohistochemical stain, and Western blot analysis, we demonstrated that the levels of procollagen type I mRNA and protein in photoaged and naturally aged human skin *in vivo* are significantly lower than those of young skin. Furthermore, we demonstrated, by northern blot analysis, that the procollagen α1(I) mRNA expression of photoaged skin is much greater than that of sun-protected skin in the same individual. *In situ* hybridization and immunohistochemical stain were used to show that the expression of type I procollagen mRNA and protein in the fibroblasts of photoaged skin is greater than for naturally aged skin. In addition, it was found, by Western blot analysis using protein extracted from the dermal tissues, that the level of procollagen type I protein in photoaged skin is lower than that of naturally aged skin. The level of matrix metalloproteinase-1 protein and the activity of matrix metalloproteinase-2 were higher in the dermis of photoaged skin than in naturally aged skin. Our results suggest that the natural aging process decreases collagen synthesis and increases the expression of matrix metalloproteinases, whereas photoaging results in an increase of collagen synthesis and greater matrix metalloproteinase expression in human skin *in vivo*. Thus, the balance between collagen synthesis and degradation leading to collagen deficiency is different in photoaged and naturally aged skin. ***Key words: aging/collagen/human skin/MMP/photoaging. J Invest Dermatol 117:1218–1224, 2001***

The aging process of the skin can be divided into intrinsic aging and photoaging. Damage to human skin due to repeated exposure to ultraviolet (UV) radiation from the sun (photoaging) and damage occurring because of the passage of time (chronologic aging) are considered distinct entities rather than similar skin aging processes. Clinically, naturally aged skin is smooth, pale, and finely wrinkled. In contrast, photoaged skin is coarsely wrinkled and associated with dyspigmentation and telangiectasia. The most dramatic histologic differences between intrinsic aging and photoaging occur within the dermis (Lavker, 1979; Lavker and Kligman, 1988; Gilchrest, 1989).

Alterations in collagen, the major structural component of skin, have been suggested as a cause of the clinical changes observed in photoaged and naturally aged skin (Fisher *et al*, 1997; Varani *et al*, 2000). The dermis contains predominantly type I collagen (85%–90%) with lesser amounts of type III collagen (10%–15%). Dermal fibroblasts synthesize the individual polypeptide chains of types I and III collagen as precursor molecules called procollagen (Smith *et al*, 1986). During the formation of insoluble collagen fibrils, specific proteases cleave the carboxy and amino terminal domains, giving rise to pN collagen (procollagen from which the carboxy terminal propeptide has been cleaved) and pC collagen (procollagen from which the amino terminal propeptide has been cleaved), respectively. Because type I and type III procollagen, pN collagens, and pC collagens are precursor molecules of mature collagen, their levels generally reflect the level of collagen biosynthesis (Haukipuro *et al*, 1991; Oikarinen *et al*, 1992).

Fisher *et al* (1996; 1997) have shown that UV irradiation induces the synthesis of matrix metalloproteinases (MMP) in human skin *in vivo*. They proposed that MMP-mediated collagen destruction accounts, in large part, for the connective tissue damage that occurs in photoaging. In addition, the same group of investigators reported that type I and type III procollagen levels are significantly lower in severely photodamaged human skin. Thus, they claimed that collagen synthesis is reduced more in photoaged human skin than in naturally aged skin *in vivo* (Talwar *et al*, 1995). Recently, it has been suggested that collagen damage due to natural skin aging may arise, as it does in photoaging, from elevated MMP expression with a concomitant reduction in collagen synthesis. Varani *et al* (2000) reported that with increasing age MMP levels become higher and collagen synthesis becomes lower in sun-protected human skin *in vivo*.

There has been no human study to compare the collagen metabolism directly between photoaged and naturally aged skin. In

Manuscript received February 19, 2001; revised June 7, 2001; accepted for publication July 11, 2001.

Reprint requests to: Dr. Hee Chul Eun, Department of Dermatology, Seoul National University Hospital, 28 Yongon-dong, Chongno-Gu, Seoul 110-744, Korea. Email: hceun@snu.ac.kr

Abbreviation: MMP, matrix metalloproteinase.

this study, we investigated and compared the expressions of type I procollagen mRNA and protein, the level of MMP-1 protein, and 72 kDa (MMP-2) and 92 kDa (MMP-9) gelatinase activities in sun-exposed and sun-protected skin of young and old subjects. We confirmed that, in chronologically aged skin, collagen synthesis was lower and MMP expression was higher. In photoaged skin, however, chronic exposure to UV radiation may stimulate collagen synthesis and increase MMP expression directly or indirectly in the dermal fibroblasts of human skin *in vivo*. Thus, the balance between collagen synthesis and degradation leading to collagen deficiency in photoaging and natural skin aging may be different.

MATERIALS AND METHODS

Skin samples A total of 14 young Koreans (11 men and three women, mean age 21.8 y, age range 19–29 y) and 16 elderly Koreans (11 men and five women, mean age 70.9 y, age range 60–87 y), four of whom were in their 60s with severe photodamage, without current or prior skin disease, provided both upper-inner arm and forearm skin samples. All elderly subjects had severely photodamaged skin, which was more than grade 5 by our photographic photodamage scales (Chung *et al*, 2001). Either 6 mm or 8 mm punch biopsy specimens were obtained from photodamaged extensor forearm skin and from sun-protected upper-inner arm and buttock skin. Another group of volunteers (12 men and nine women, three subjects in each decade of age from the 20s to 80s) provided both buttock and facial (crow's feet area) skin samples. Two millimeter punch biopsy specimens were obtained from the face.

The specimens for Western blot and northern blot analysis were snap frozen in liquid nitrogen, and the specimens for immunohistochemical stain were oriented immediately into a cryomatrix (Shandon, Pittsburgh, PA) and stored at –70°C. For the Western blot analysis and zymography, we used the protein extracted from the dermis. To separate the dermis from the epidermis, the defrosted skin samples were heated at 55°C for 2 min and then separated gently into epidermis and dermis with forceps. Each sample of dermis was homogenized in lysis buffer (containing 1% Triton X-100, 150 mM NaCl, 5 mM ethylenediamine tetraacetic acid, 0.1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg per ml aprotinin, and 2 μg per ml leupeptin). Lysates were rotated at 4°C for 15 min and centrifuged at 13,000*g* for 15 min, and the supernatant was used for Western blot analysis and zymography. This study was approved by the Institutional Review Board at the Seoul National University Hospital, and all subjects gave written informed consent.

Northern blot analysis The total RNA was isolated from the whole punch-biopsied skin samples using a Trizol reagent (Gibco BRL, Gaithersburg, MD) following the manufacturer's instructions. The RNA was electrophoresed through a 0.8% formaldehyde gel and transferred onto a Hybond membrane (Amersham, Arlington Heights, IL) using a turboblotter (Schleicher and Schuell, Keene, NH) downward capillary transfer system. The cDNA probes were prepared by labeling the 366 bp Xho I–Pvu II fragment of procollagen α1(I) and the 0.7 kb fragment of 36B4 (36B4 encodes a ribosomal protein and was used as an internal control) with [α-^{32}P]dCTP with a Prime-It II kit (Stratagene, La Jolla, CA). The blots were exposed to a Fuji Imaging Plate (BAS-2500, Fujifilm, Japan) and quantitated by using a densitometry program (Raytest Isotopenmeßgerate, Germany). After normalizing for lane loading, as determined by 36B4 intensity, the percentage increases in the mRNA transcripts were determined.

***In situ* hybridization** The 366 bp Xho I–Pvu II fragment of procollagen α1(I) was cloned into pCR2.1-TOPO. Digoxigenin-containing sense and antisense riboprobes that detect human procollagen α1(I) were synthesized using T7 RNA polymerase. *In situ* hybridization was performed on 8 μm sections as described in detail elsewhere (Fisher *et al*, 1997). All samples were treated with proteinase K and were washed in a 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. After hybridization at 52°C, the slides were washed under stringent conditions, including treatment with RNase A to remove the unhybridized probe. Hybridization signals were detected immunohistochemically with the use of an alkaline phosphatase-conjugated antidigoxigenin antibody. Entire fields in each section were examined at 400× magnification, the number of positive fibroblasts in the papillary dermis was counted, and the mean per 400× field was calculated.

Immunohistochemical staining Serial sections of 8 μm thickness were mounted onto silane-coated slides (Dako, Glostrup, Denmark). The acetone-fixed frozen sections were stained with monoclonal antihuman procollagen type I C-peptide (PIC) antibody (Takara, Shiga, Japan), and monoclonal antiprocollagen type I aminoterminal extension peptide (SP1.D8) antibody (Developmental Studies Hybridoma Bank, Iowa City, IA), diluted 1:1600, was applied for 1 h at room temperature. After rinsing in phosphate-buffered saline, the sections were visualized by means of an LSAB kit (Dako) that employed a biotinylated secondary antibody and horseradish–streptavidin conjugate. 3-Amino-9-ethylcarbazole was used as a chromogenic substrate. The sections were

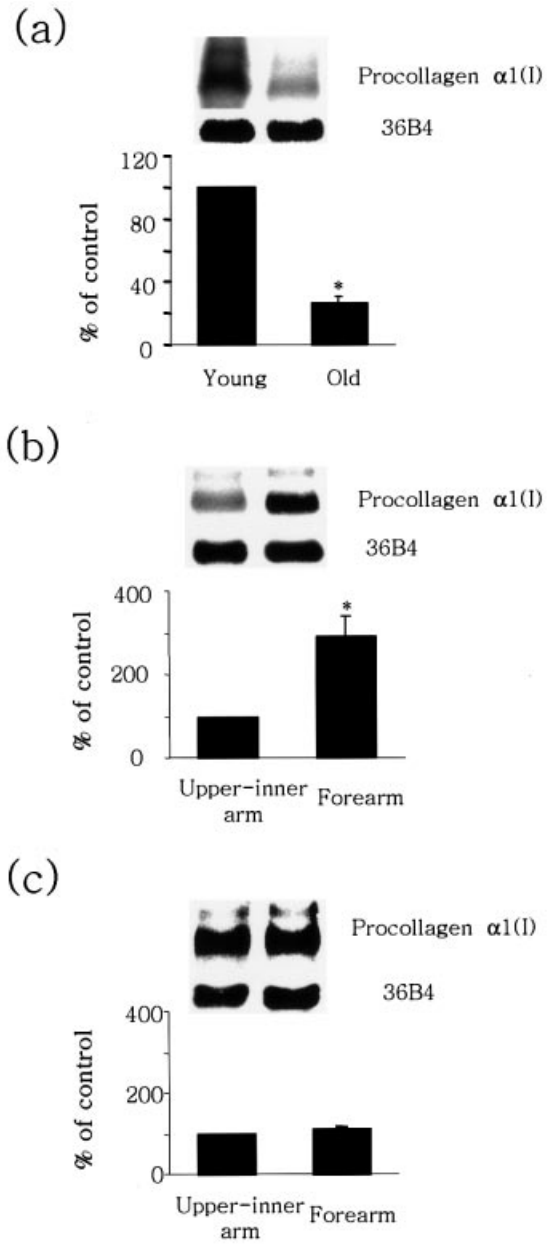

Figure 1. Procollagen α1(I) mRNA expression was increased in chronically photodamaged human skin *in vivo* compared with naturally aged skin. (*a*) The procollagen α1(I) mRNA level was measured in the total RNA extracted from the buttock skin of young ($n = 4$) and old ($n = 4$) subjects by northern blot. *$p < 0.05$, old skin *versus* young skin for procollagen α1(I) mRNA, mean ± SEM. (*b*) The procollagen α1(I) mRNA level was measured in the total RNA extracted from the upper-inner arm and forearm skin of the same elderly ($n = 4$) subjects by northern blot. *$p < 0.05$, upper-inner arm *versus* forearm skin, mean ± SEM. (*c*) The procollagen α1(I) mRNA level was measured in the total RNA extracted from the upper-inner arm and forearm skin of the same young ($n = 4$) subjects by northern blot. *$p < 0.05$, upper-inner arm *versus* forearm skin of young subjects, mean ± SEM.

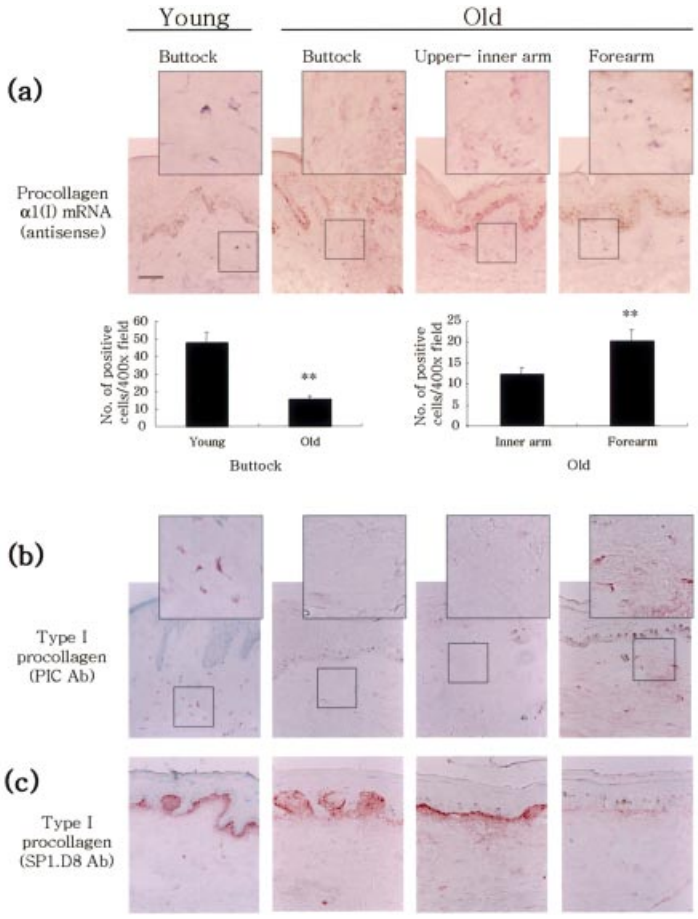

Figure 2. The expressions of type I procollagen mRNA and protein were higher in photoaged human skin *in vivo* than in chronologically aged skin. (*a*) Procollagen α1(I) mRNA in the fibroblasts was detected by *in situ* hybridization. The number of positive fibroblasts in the papillary dermis was counted as described in *Methods*. **p < 0.01. Values are the mean ± SEM per 400× field of five subjects. (*b*) The intracellular expression of type I procollagen protein in the fibroblasts was detected with PIC antibody, and (*c*) the extracellular expression of type I procollagen was detected with SP1.D8 antibody by immunohistochemical staining. The photographs are representative of young ($n = 5$) and old ($n = 5$) subjects, respectively. Note the melanin pigments in the basal cell layer of the epidermis. Areas outlined in boxes are shown in 2.5-fold enlargements. *Scale bar*: 25 μm.

counterstained briefly in Mayer's hematoxylin. Entire fields in each section were examined at 400× magnification, the number of positive fibroblasts in the papillary dermis was counted, and the mean per 400× field was calculated. Control staining was performed with normal rabbit and mouse immunoglobulin and showed no immunoreactivity (data not shown).

Western blot analysis Procollagen levels in the soluble protein extracted directly from the dermal tissues of the punch-biopsied skin samples were determined with a monoclonal antiprocollagen type I aminoterminal extension peptide (SP1.D8) antibody (Developmental Studies Hybridoma Bank) by Western blot analysis, as described previously (Chung *et al*, 2000).

Zymography The gelatinolytic activity was analyzed by gelatin zymography using zymogram gels (Novex, San Diego, CA) containing 0.1% gelatin as substrate. The proteins extracted from the dermal tissue were separated on zymogram gel. The gels were renatured in 2% Triton X-100, 50 mM Tris–HCl, pH 7.4, and then incubated in an enzyme substrate buffer (50 mM Tris–HCl, pH 8.0, 5 mM $CaCl_2$, 0.02% NaN_3) overnight at 37°C. The gels were stained with Coomassie Blue R250 and destained in water. The unstained areas corresponding to gelatinolytic activity were quantified by image analyzer. Zymography was performed at least twice for each sample.

Statistical analysis Statistical analyses were performed using the Mann–Whitney *U* test and Kruskal–Wallis test. A p-value of less than 0.05 was considered statistically significant. All analyses were performed with Statistical Analysis Software (SAS, Cary, NC).

RESULTS

The expression of procollagen α1(I) mRNA was increased in photoaged skin compared with sun-protected human skin *in vivo* To investigate the effect of aging and photoaging on collagen synthesis in human skin *in vivo*, procollagen α1(I) mRNA was measured by northern blot analysis using total RNA extracted directly from the punch-biopsied specimens of sun-protected (buttock and upper-inner arm) and sun-exposed (forearm) skin of both young and elderly people. We demonstrated that procollagen α1(I) mRNA in the buttock skin of elderly subjects ($n = 4$) was lower by an average of 74% than in the buttock skin of young subjects ($n = 4$, **Fig 1*a***). Unexpectedly, the forearm skin of the elderly ($n = 4$) showed an expression for procollagen α1(I) mRNA that was 2.9 times greater on average than for the upper-inner arm skin of the same individuals (**Fig 1*b***). To confirm that this difference was not due to differences in body site, we investigated the expression of procollagen α1(I) mRNA in the forearm and upper-inner arm of the young subjects ($n = 4$, **Fig 1*c***). Because the level of procollagen α1(I) mRNA was similar in both areas of the young subjects, we concluded that this increased expression of procollagen α1(I) mRNA in the photoaged skin of elderly subjects, relative to naturally aged skin, may be the result of chronic sun exposure.

The number of procollagen α1(I) mRNA-positive fibroblasts in photodamaged skin was higher than in the sun-protected skin of the same individuals To localize the expression of procollagen α1(I) mRNA in human skin *in vivo*, sun-exposed (forearm) and sun-protected (upper-inner arm) skin samples ($n = 5$) were taken by punch biopsy, and analyzed for procollagen α1(I) mRNA by *in situ* hybridization. We found that there were few fibroblasts expressing procollagen mRNA in the aged sun-protected (buttock and upper-inner arm) skin, whereas there were many procollagen mRNA-positive fibroblasts in the young (buttock) skin (**Fig 2*a***). We also demonstrated that the number of fibroblasts expressing procollagen α1(I) mRNA in the papillary dermis of photoaged (forearm) skin (20.4 ± 2.5 per 400× magnified microscopic field, mean ± SEM, $n = 5$) of the elderly was higher than in the sun-protected (upper-inner arm) skin

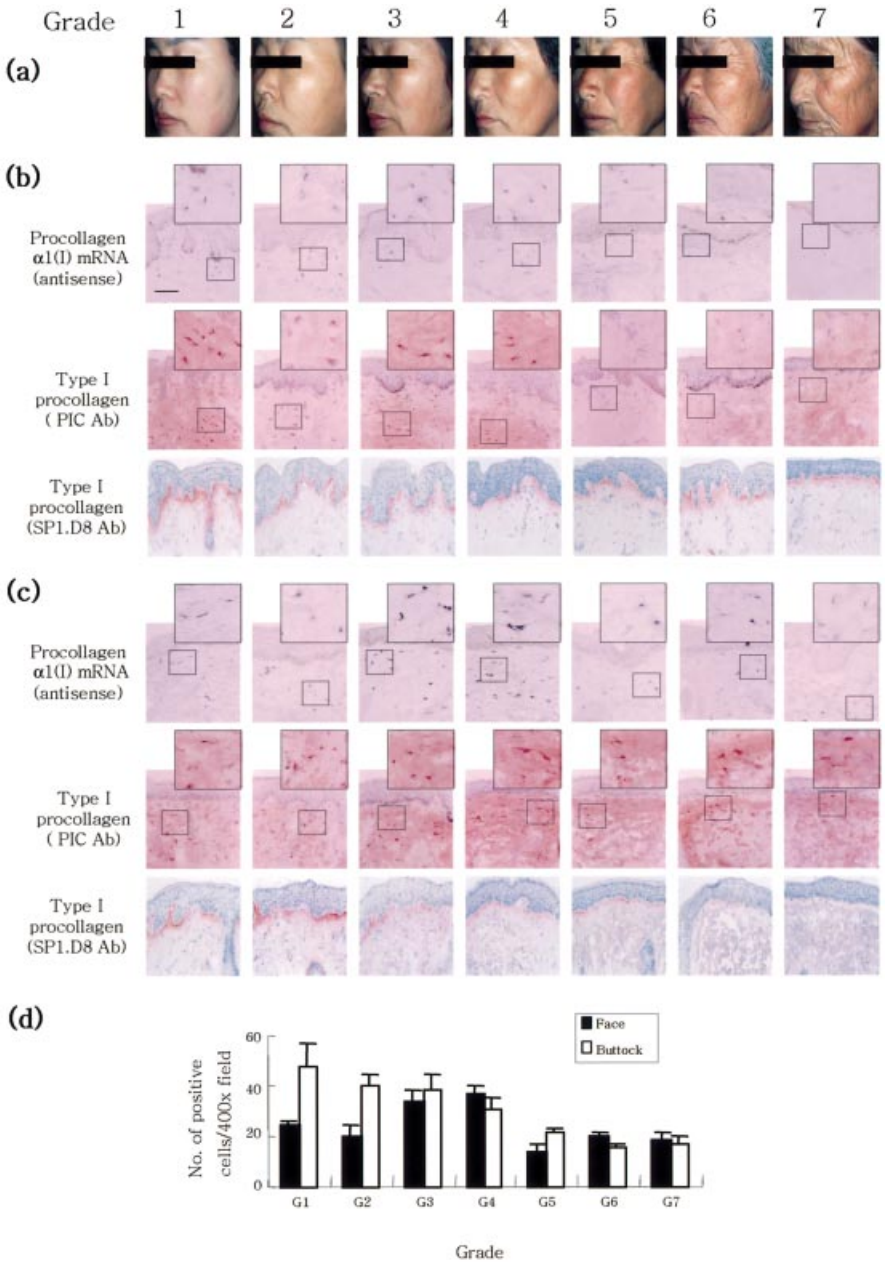

Figure 3. Aging- and photoaging-dependent changes of procollagen α1(I) mRNA and protein expression in human skin *in vivo*. (*a*) The grades of photoaging in Korean subjects. (*b*) Sun-protected (buttock) and (*c*) sun-exposed (face) skin samples were taken from the same subject. Three subjects in each group (total seven groups) were studied. Procollagen α1(I) mRNA in the fibroblasts was detected by *in situ* hybridization. The intracellular expression of type I procollagen protein in the fibroblasts was detected with PIC antibody, and the extracellular expression of type I procollagen was detected with SP1.D8 antibody by immunohistochemical staining. (*d*) The number of fibroblasts expressing procollagen mRNA in the papillary dermis was counted. Comparison of the number of fibroblasts (at face and buttock) for each wrinkle grade by Kruskal–Wallis test, $p < 0.001$. Values are the mean ± SEM per 400× field of three subjects. The photographs of each grade are representative of three subjects. Note the melanin pigments in the basal cell layer of the epidermis. Areas outlined in boxes are shown as 2.5-fold enlargements. *Scale bar*: 25 μm.

(12.4 ± 1.5, $n = 5$) (**Fig 2*a***). No difference was observed with respect to the expression of procollagen α1(I) mRNA between upper-inner arm and forearm young skin (data not shown).

Intracellular and extracellular expression of type I procollagen protein in aged and photoaged human skin *in vivo* To detect and compare the expression of type I procollagen protein in both aged and photoaged skin ($n = 5$), two kinds of antibodies for type I procollagen were used in this study. The antihuman procollagen type I C-peptide (PIC) antibody showed strong immunoreactivity for intracellular procollagen proteins (**Fig 2*b***), whereas the antiprocollagen type I amino-terminal extension peptide (SP1.D8) antibody stained the extracellular procollagen proteins, especially beneath the dermo-epidermal junction (**Fig 2*c***). The intracellular expression of procollagen protein in the fibroblasts of young buttock skin was greater than for aged buttock skin with the PIC antibody (**Fig 2*b***). We demonstrated that the number of procollagen-positive fibroblasts in the upper dermis of photoaged (forearm) skin (19.3 ± 0.8 per 200× magnified microscopic field, mean ± SEM, $n = 5$) was higher than in the sun-protected (upper-inner arm) skin (1.6 ± 1.7, $n = 5$) of the same individuals (**Fig 2*b***). No difference was apparent in the expression of procollagen protein between upper-inner arm and forearm young skin (data not shown).

Immunostaining with the SP1.D8 antibody of young buttock skin ($n = 5$) showed greater expression of type I procollagen protein beneath the dermo-epidermal junction than old buttock skin ($n = 5$) (**Fig 2*c***). In contrast to the intracellular expression of type I procollagen protein, the extracellular expression in the papillary dermis of photoaged skin was lower than that of sun-protected skin of the same individuals (**Fig 2*c***).

The expression of type I procollagen mRNA and protein gradually decreased during the natural aging process, but tended to be unaffected by the photoaging process To confirm our results and to investigate the changes in collagen metabolism caused by natural aging and photoaging over a lifetime, both sun-exposed (crow's feet area) and sun-protected (buttock) skin samples ($n = 3$) were taken from individuals belonging to

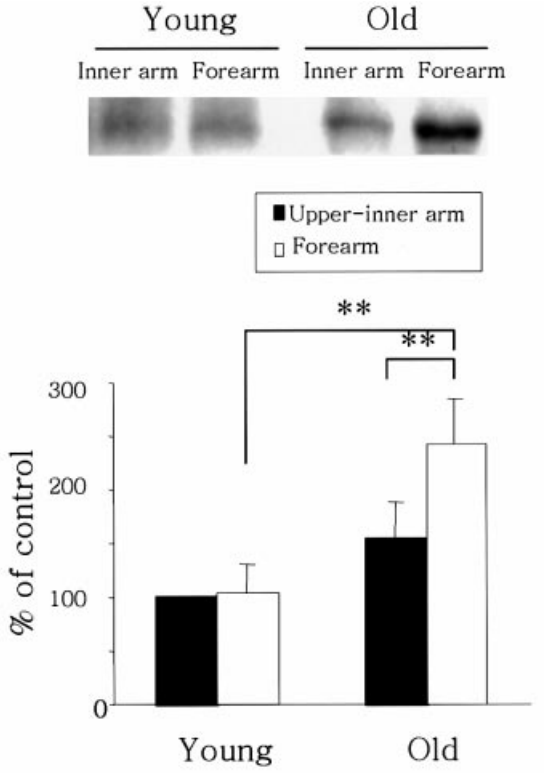

Figure 4. The level of MMP-1 protein was higher in the dermis of photoaged human skin *in vivo*. The MMP-1 protein was measured in soluble proteins extracted from the dermis of the upper-inner arm and forearm skin of young ($n = 5$) and old ($n = 7$) subjects, respectively, by Western blots. **$p < 0.01$, old skin *versus* young skin; **$p < 0.01$, forearm *versus* upper-inner arm, mean ± SEM.

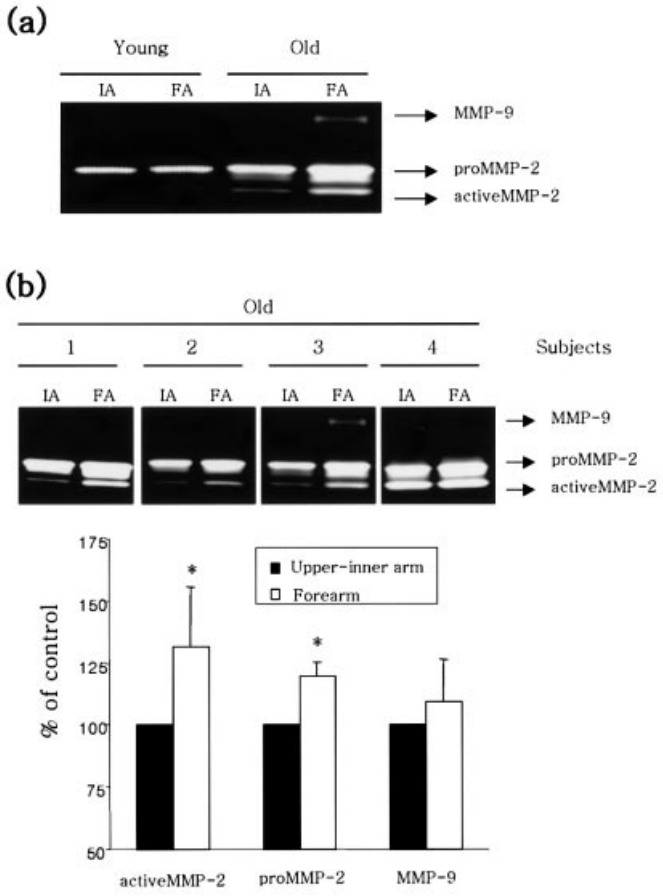

Figure 5. 72 kDa gelatinase (MMP-2) activity was increased in the dermis of photoaged human skin *in vivo* compared with naturally aged skin. (*a*) Gelatinase activities in young and old subjects were analyzed by gelatinase zymography. This figure is representative of findings in five young and five old subjects. (*b*) The 72 kDa and 92 kDa gelatinase activities in the dermis of upper-inner arm and forearm of old ($n = 5$) subjects were analyzed by gelatin zymography. *$p < 0.05$, forearm *versus* upper-inner arm skin of old subjects, mean ± SEM. IA, inner arm; FA, forearm.

seven different age groups (**Fig 3*a***, each decade from 20s to 80s). These samples were used for *in situ* hybridization of procollagen α1(I) mRNA and immunostaining of type I procollagen. Buttock (sun-protected) skin showed a gradual decrease in type I procollagen mRNA and intracellular procollagen (detected by PIC antibody) expression. Moreover, there were very few fibroblasts expressing mRNA and intracellular proteins in naturally aged skin above the age of 60 (grade 5) (**Fig 3*b***). Nevertheless, we observed extracellular procollagen protein, as detected by SP1.D8 antibody, in naturally aged skin above the age of 60 (**Fig 3*b***). Facial (sun-exposed) skin showed a tendency to maintain the expression of type I procollagen mRNA and intracellular protein (detected by PIC antibody) even after the 60s to over 80s (**Fig 3*c***). We also demonstrated that the number of fibroblasts expressing type I procollagen mRNA in facial 40- and 50-y-old skins was significantly higher than that of 20- and 30-y-old skins (**Fig 3*d***). Because there was an extensive accumulation of elastotic materials in the upper dermis from grade 5, we were able to detect fibroblasts only in the papillary dermis and not in the area of solar elastosis. The expression of extracellular type I procollagen protein detected by SP1.D8 antibody gradually decreased and became undetectable at grade 5 (**Fig 3*c***), although there was still a significant amount of type I procollagen synthesis.

The level of MMP-1 protein in the dermis of photoaged skin was higher than that in sun-protected skin of the same individuals We compared the expression of MMP-1 protein in the dermis of sun-exposed and sun-protected skin of both young ($n = 5$) and elderly ($n = 7$) subjects (**Fig 4**). The level of MMP-1 expression between the upper-inner arm and forearm was the same in young skin. In both the naturally aged and photoaged skin of the elderly, the expressions of MMP-1 protein were higher than in the sun-protected skin of young people, by an average of 55% ($p > 0.05$) and 142% ($p < 0.01$), respectively, even though only the photoaged skin showed a significant increase. The photodamaged (forearm) skin of the elderly showed a greater increase in MMP-1 protein expression (1.7-fold, $p < 0.01$) compared with that of the sun-protected skin of the same individuals.

MMP-2 (72 kDa gelatinase) was higher in the dermis of photoaged skin than naturally aged skin We investigated the activities of gelatinase (92 kDa and 72 kDa) in the dermis of the sun-exposed and sun-protected skin of both young ($n = 5$) and elderly ($n = 5$) subjects. We demonstrated that MMP-2 and MMP-9 tended to be higher by more than 1.5 times in aged and photoaged skin in comparison with young skin (**Fig 5*a***). There was a significant difference of active MMP-2 (132%, $p < 0.05$) and pro-MMP-2 (120%, $p < 0.05$) between the aged and photoaged skin of the elderly. There were no significant differences in the activity of MMP-9 between the sun-exposed and sun-protected skin of old subjects (**Fig 5*b***).

Net amount of type I procollagen protein in the dermis of photoaged skin was lower than that in sun-protected skin We performed Western blot analysis for type I procollagen using the total protein extracted from the dermal tissue of the sun-exposed and sun-protected skin of both young ($n = 5$) and old

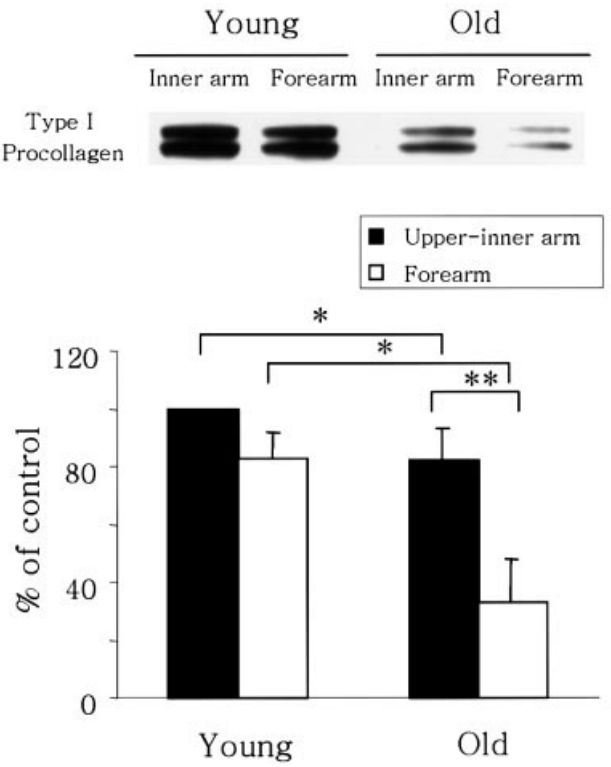

Figure 6. Net amount of type I procollagen protein in photoaged skin was lower than in naturally aged skin. Type I procollagen protein was measured in soluble total protein extracted from the dermal tissues of the upper-inner arm and forearm of young ($n = 5$) and old ($n = 7$) subjects by Western blots. *$p < 0.05$, old skin *versus* young skin; **$p < 0.01$, forearm *versus* upper-inner arm, mean ± SEM.

($n = 7$) persons. The photoaged (forearm) skin of the elderly showed less type I procollagen protein compared with naturally aged (upper-inner arm) skin by an average of 43% ($p < 0.01$, $n = 7$, **Fig 6**). The levels of type I procollagen between the forearm and upper-inner arm of young subjects, however, were similar ($n = 5$, **Fig 6**). Because the level of procollagen protein was similar between forearm and upper-inner arm of young subjects, we concluded that this decreased expression of procollagen protein in the photoaged skin of elderly subjects, compared with naturally aged skin, could be the result of increased degradation by MMP induced by chronic UV exposure.

DISCUSSION

In this study, we investigated the modulation of procollagen metabolism between aged and photoaged human skin *in vivo*. The balance between the synthesis of procollagen and degradation of newly synthesized procollagen is important for determining the net amount of collagen synthesis in human skin *in vivo*. We demonstrated clearly in our study, by northern blot analysis, *in situ* hybridization, and immunohistochemical stain, that fibroblasts in the photodamaged skin of elderly subjects express more mRNA and protein of type I procollagen than those of sun-protected skin of the same individuals. Our results are different from the report by Talwar *et al* (1995), which suggested a reduced synthesis of both type I and type III procollagens in photodamaged human skin compared with sun-protected skin of the same subjects, and the degree of reduction correlated with the severity of clinical photodamage. As they mentioned in their paper, it is possible that the reduced levels of procollagen occur as a result of decreased synthesis and/or increased breakdown of type I and type III procollagen in photodamaged skin. The three methods they used in their study (radioimmunoassay, Western blot analysis, and immunohistology for collagen detection) cannot distinguish between decreased synthesis and increased breakdown of collagen for the cause of reduced procollagen levels in photodamaged skin.

Recently, it was reported that both the mRNA and the protein of type I procollagen were reduced in photodamaged skin (forearm) compared with the sun-protected skin (buttock) of the same individual, by *in situ* hybridization and immunostaining, respectively (Varani *et al*, 2001). We do not know the reasons for this discrepancy. In their study, Varani and coworkers compared sun-exposed forearm skin with sun-protected hip skin of elderly persons. They did not exclude the possibility, however, that hip skin may produce more procollagen constitutively than forearm skin. We confirmed that the forearm and upper-inner arm skins of the young donors used in our study showed the same levels of procollagen mRNA by northern blot analysis (**Fig 1*c***), and also observed serial changes of procollagen mRNA expression in buttock and facial skins with aging (**Fig 2**), which supports our findings.

On the other hand, we demonstrated that the dermis of photoaged skin of elderly people expressed much more MMP-1 protein and MMP-2 activity than the sun-protected skin of the same subjects. These higher expressions of MMP-1 and MMP-2 in the dermis degrade the newly synthesized procollagen secreted from the fibroblasts, and thereby lead to reduced procollagen expression in photoaged human skin *in vivo*. It has been known that UV radiation induces the synthesis of MMP from keratinocytes, fibroblasts, and inflammatory cells (Woodley *et al*, 1986; Lavker and Kligman, 1988; Scharffetter *et al*, 1991; Fisher *et al*, 1996; 1997). The increased degradation of procollagen by the MMP may contribute to the observed reductions in type I and type III collagen precursors in photodamaged skin (Oikarinen and Kallioinen, 1989; Talwar *et al*, 1995). As demonstrated in this study by Western blot analysis, the net effects of the increase of procollagen synthesis and greater degradation of procollagen by a higher amount of MMP in photoaged skin *in vivo* may cause a severe deficiency of procollagen.

The reasons why fibroblasts in chronically photodamaged skin express more procollagen mRNA and proteins still need to be investigated. Kligman *et al* (1989) also demonstrated that chronic UV exposure resulted in increased total collagen synthesis in hairless mouse skin at 4–16 wk, returning to control levels at 20 wk. This increase in total collagen contents may be due to a proportional increase in both type I and type III collagen. In addition, they also suggested that the decrease in total collagen beginning at week 20 may be the result of enzymatic digestion by collagenase secreted by cells of the inflammatory infiltrate, and the degradation of collagen may finally exceed the capacity of the UV-irradiated fibroblasts to synthesize new collagen.

Recently, Starcher *et al* (1999) also demonstrated, in hairless mice by *in situ* hybridization, that collagen mRNA levels were greatly enhanced in response to chronic UV radiation and that this increase was correlated with the presence of inflammatory cells. They suggested that presumably the inflammatory cells were releasing factors that stimulate the fibroblasts to upregulate collagen synthesis. They also found that, even though an enhanced collagen message persisted for the duration of the irradiation period, total hydroxyproline was not increased following irradiation and there tended to be a slight decrease. Their results suggested that either the elevated message levels did not translate into increased collagen production or proteolytic enzymes degraded the collagen as fast as it was produced.

It has been reported that the fibroblasts derived from patients who received long-term psoralen plus UVA treatment contained higher levels of collagen and elastin mRNA than control cells (Oikarinen, 1990). This suggests that, during actinic damage, there may be a repair process in which fibroblasts actively synthesize a connective tissue component, such as collagen and elastin (Oikarinen, 1990). In this study, the elevated number of fibroblasts expressing procollagen mRNA in 40- and 50-y-old facial skins suggests that the UV-induced damage repair process in human skin may peak at these ages.

Previously, we demonstrated that cultured human photoaged dermal fibroblasts showed enhanced proliferation and collagen synthesis compared with aged fibroblasts from the same individuals (Chung *et al*, 1996). UV radiation stimulates and activates various cells to produce and release cytokines that may play a significant role in the process of photoaging (Kondo, 2000). It is known that the inflammatory infiltrates in evolving photoaged skin are responsible for producing various cytokines, such as tumor necrosis factor α and transforming growth factor β, which may inhibit or stimulate the production of a dermal extracellular matrix, thereby leading to the characteristic changes seen in photoaging (Oikarinen, 1990; Griffiths and Voorhees, 1993; Werth *et al*, 1997).

In the naturally aged skin of old subjects in comparison with young skin, we demonstrated that the expression of procollagen α1(I) mRNA was lower, and that the level of MMP-1 and the activities of MMP-2 and MMP-9 tended to be higher, in naturally aged human skin *in vivo*. Recently, Varani *et al* (2000) also demonstrated that collagen synthesis was decreased in the 80+ age group, and the total levels of MMP-1, MMP-2, and MMP-9 were elevated compared with the 18–29-y-old group in human sun-protected skin *in vivo*. Thus, less synthesis of procollagen and more degradation by increased MMP would result in collagen deficiency in the intrinsically aged skin of the elderly.

The findings presented here indicate that there is a significant difference in the pathomechanisms leading to collagen deficiency between chronologic aging and photoaging. Naturally aged skin showed a reduced synthesis of collagen and an elevated expression of MMP. On the other hand, in photoaging, collagen synthesis is induced by chronic UV irradiation but the degradation of collagen by the many more MMP in photodamaged skin will be greater. Thus, the net effect of synthesis and degradation is shifted to a negative balance leading to collagen deficiency in the chronically photodamaged skin of the elderly.

This work was supported by a grant 'Life Phenomena and Function Research Group Program (2000)', from the Ministry of Science and Technology, and a research agreement with Pacific Corporation. We thank Mi Ran Kwon for excellent technical assistance.

REFERENCES

Chung JH, Youn SH, Kwon OS, *et al:* Enhanced proliferation and collagen synthesis of human dermal fibroblasts in chronically photodamaged skin. *Photodermatol Photoimunol Photomed* 12:84–89, 1996

Chung JH, Kang S, Varani J, Lin J, Fisher GJ, Voorhees JJ: Decreased extracellular-signal regulated kinase and increased stress-activated MAP kinase activities in aged human skin *in vivo*. *J Invest Dermatol* 115:177–182, 2000

Chung JH, Lee SH, Youn CS, *et al:* Cutaneous photodamage in Koreans: influence of sex, sun-exposure, smoking and skin color. *Arch Dermatol* 137:1043–1051, 2001

Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, Voorhees JJ: The molecular basis of sun-induced premature skin aging and retinoid antagonism. *Nature* 379:335–338, 1996

Fisher GJ, Wang Z-Q, Datta SC, Varani J, Kang S, Voorhees JJ: Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337:1419–1428, 1997

Gilchrest BA: Skin aging and photoaging: an overview. *J Am Acad Dermatol* 21:610–613, 1989

Griffiths CEM, Voorhees JJ: Topical retinoic acid for photoaging. Clinical response and underlying mechanisms. *Skin Pharmacol* 6 (Suppl. 1):70–77, 1993

Haukipuro K, Meikko J, Risteli L, Kairaluoma MI, Risteli J: Synthesis of type I collagen in healing wounds in humans. *Ann Surg* 213:75–80, 1991

Kligman LH, Gebre M, Alper RA, Kefalides NA: Collagen metabolism in ultraviolet irradiated hairless mouse skin and its correlation to histochemical observations. *J Invest Dermatol* 93:210–214, 1989

Kondo S: The roles of cytokines in photoaging. *J Dermatol Sci* 23:s30–s36, 2000

Lavker RM: Structural alterations in exposed and unexposed aged skin. *J Invest Dermatol* 73:559–566, 1979

Lavker RM, Kligman AM: Chronic heliodermatitis: a morphologic evaluation of chronic actinic dermal damage with emphasis on the role of mast cells. *J Invest Dermatol* 90:325–330, 1988

Oikarinen A: The aging of skin: chronoaging versus photoaging. *Photodermatol Photoimmunol Photomed* 7:3–4, 1990

Oikarinen A, Kallioinen M: A biochemical and immunohistochemical study of collagen in sun-exposed and protected skin. *Photodermatol Photoimmunol Photomed* 6:24–31, 1989

Oikarinen A, Autio P, Kiistala U, Risteli L, Risteli J: A new method to measure type I and III collagen synthesis in human skin *in vivo*: demonstration of decreased collagen synthesis after topical glucocorticoid treatment. *J Invest Dermatol* 98:220–225, 1992

Scharffetter K, Wlaschek M, Hogg A, *et al:* UVA irradiation induces collagenase in human dermal fibroblasts *in vitro* and *in vivo*. *Arch Dermatol Res* 283:506–511, 1991

Smith LT, Holbrook KA, Madri JA: Collagen types I, III, and V in human embryonic and fetal skin. *Am J Anat* 175:507–521, 1986

Starcher B, Pierce R, Hinek A: UVB irradiation stimulates deposition of new elastic fibers by modified epithelial cells surrounding the hair follicles and sebaceous glands in mice. *J Invest Dermatol* 112:450–455, 1999

Talwar HS, Griffiths CEM, Fisher GJ, Hamilton TA, Voorhees JJ: Reduced type I and type III procollagens in photodamaged adult human skin. *J Invest Dermatol* 105:285–290, 1995

Varani J, Warner RL, Gharaee-Kermani M, *et al:* Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. *J Invest Dermatol* 114:480–486, 2000

Varani J, Spearman D, Perone P, et al: Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen *in vitro*. *Am J Pathol* 158:931–942, 2001

Werth VP, Williams KJ, Fisher EA, Bashir M, Rosenbloom J, Shi X: UVB irradiation alters cellular responses to cytokines: role in extracellular matrix gene expression. *J Invest Dermatol* 108:290–294, 1997

Woodley DT, Kalebec T, Banes AJ, Link W, Prunieras M, Liotta L: Adult human keratinocytes migrating over nonviable dermal collagen produce collagenolytic enzymes that degrade type I and type IV collagen. *J Invest Dermatol* 86:418–423, 1986