Gelatinase, a Possible Etiologic Factor of Photoaging, is Present in Healthy Human Facial Skin and is Inhibited by Turmeric Extract

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Summary

Influence of gelatinase on basement membrane (BM) structure was investigated by using a skin equivalent (SE) model. The results showed that (1) gelatinase produced by cells degraded the BM and (2) the addition of matrix metalloproteinase-specific inhibitor to the SE medium accelerated the formation of BM structure, indicating that gelatinase is involved in BM impairment. The activity of gelatinase was also studied in healthy human facial skin tissues. The result of *in situ* zymography revealed gelatinase activity around the basal layer of the epidermis, where BM integrity was severely compromised. Therefore, this enzyme was suggested to be associated with BM decomposition in human facial skin.

To assess the behavior of gelatinase in stratum corneum (SC) non-invasively, an immunological study was performed. Since positive immunostaining of pro-gelatinase B was observed in SC stripped from sun-exposed skin, whereas no positive staining detected in SC of non-irradiated skin, gelatinase in the epidermis could be non-invasively detected by measuring gelatinase in SC. Gelatinase in SC of healthy female volunteers was monitored using a special film that sensitively and conveniently detects gelatinase. Ninety percent of SC from facial skin (100 women, 40's-50's) was gelatinase-positive. On the other hand, SC from non-irradiated skin was negative. These results strongly suggest that (1) gelatinase is constantly produced in the facial epidermis of most middle-aged woman during their daily life, and (2) the enzyme might be involved in the aging-related degeneration of both BM and the matrix fibers of the upper layer of the dermis, acting as a very important aging factor.

Strong inhibitory activity against gelatinase was found in turmeric extract and identified curcumin as the major ingredient. Topical application of cream containing turmeric extract significantly decreased the number of gelatinase-positive SC clusters in

human facial skins. These results indicated that turmeric is an effective ingredient to prevent skin from photoaging by suppressing chlonically upregulated gelatinase activity by UV and to improve skin condition.

Introduction

Symptoms of cutaneous aging, such as wrinkles and slackness, develop earlier in facial skin than in unexposed skin. This phenomenon is known as photoaging and is characterized by various histological changes, including damage to collagen fibers, excessive deposition of abnormal elastic fibers, increase of glycosaminoglycans, and reduplication of basement membrane (BM) [1-4]. The reduplication and disruption of BM in sun-exposed skin worsens with advancing age and is apparent even in persons in their 20's, before the symptoms of aging become conspicuous, so disordered BM is considered to be a sign of early aging [5].

Recently there has been much interest in matrix metalloproteinases (MMPs) that destroy BM constituents, such as collagen IV and laminin 5, and extracellular matrix [6]. It has been reported that MMP-1, 2, 3, and 9 are increased in experimentally UV-irradiated human skin [7, 8], and gelatinase (MMP-2 and 9) might be wrinkle-inducing factors [9]. However, the role of MMPs remains to be fully established.

In this study, we focused on gelatinase as a photoaging factor in healthy female skin. We found that gelatinase exists in stratum corneum (SC) of sunlight-exposed skin, and succeeded in evaluating the activity of this enzyme non-invasively and precisely in human female facial skin. Furthermore, we found that turmeric extract, a herbal medicine, could effectively inhibit the gelatinase activity, and we identified curcumin as an active ingredient. A topical application test of a cream containing turmeric extract was also conducted, and we here report its gelatinase-inhibitory effect in human facial skin.

Materials and Methods

Skin Equivalent (SE)

Human epidermal keratinocytes were isolated from human neonatal foreskin. The keratinocytes were grown in a modified serum-free keratinocyte growth medium (KGM) containing 0.03 mmol/L Ca^{2+}. Human dermal fibroblasts were isolated from human dermis and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. SEs were prepared according to the modified method described by Tsunenaga et al. [10]. The keratinocytes were cultured on top of a dermal equivalent consisting of type I collagen and fibroblasts in a three-dimensional fashion. The culture was lifted to the air-liquid interface and only the layer of keratinocytes was exposed to air to form a cornified layer. The medium for SE culture was prepared from a 1:1 mixture of KGM and DMEM supplemented with 5% FBS, 1.8 mmol/L Ca^{2+} (the final concentration), and 250 μmol/L 2-O-α-D-glucopyranosyl-L-ascorbic acid (AA-2G), which is a stable derivative of ascorbic acid. The medium was changed three times a week. The keratinocytes piled up to form stratified squamous epithelium within 7 days. The SEs were processed to evaluate the effects of curcumin on the deposition of BM components, such as type IV collagen.

Antibodies

Antibodies used in this study were as follows: mouse monoclonal antibodies against the α1 chain of collagen IV, JK-199; rabbit polyclonal antibodies against collagen IV (MO-S-CLIV, Cosmo Bio, Tokyo, Japan); mouse monoclonal antibody against MMP-9 (Calbiochem, San Diego, CA, USA); mouse IgG_1 (negative control, DAKO, Denmark).

Transmission Electron Microscopy

SEs were fixed by microwave irradiation with Zamboni's fixative (4% paraformaldehyde and 0.2% picric acid in 0.1 mol/L phosphate buffer, pH 7.4). Each sample was rinsed with 0.1 mol/L phosphate buffer (pH 7.4), postfixed with 1% osmium tetroxide, immersed in 1% tannic acid to enhance the electron density of extracellular matrices, dehydrated with a graded series of ethanol, and embedded in epoxy resin. Ultrathin sections were cut and stained with 5% uranyl acetate and Raynolds' lead citrate. The sections were examined with a transmission electron microscope (Hitachi H-7100, Hitachi, Tokyo).

Synthetic MMP Inhibitor

The inhibitor CGS27023A was synthesized by Shiseido Research Center [11]. Its K_i values against MMPs were as follows: MMP-1, 33 nmol/L; MMP-2, 20 nmol/L; MMP-3, 43 nmol/L; MMP-9, 8 nmol/L. In order to inhibit MMPs in SEs, CGS27023A was added to culture media at the concentration of 10 μmol/L.

Determination of Collagen Content

Collagen in culture media was hydrolyzed by heating at 110 °C for 18 h after having been mixed with an equal volume of 12 N hydrochloric acid. The hydrolysate was dried up by using a centrifugal evaporator and analyzed for hydroxyproline by amino acid analysis [12].

In situ Gelatin Zymography

In situ gelatin zymography was carried out using cross-linked gelatin membrane with a thickness of 7 μm on polyester film (FIZ-GN film, Fuji Photo Film, Tokyo), which is suitable for detecting gelatinase activity in tissues [13-15]. Each frozen tissue sample was

sliced to 5 μm sections using a cryostat (CM3050S, Leica, Nusslich, Germany) and placed on FIZ-GN film. After 18 h of incubation in a moist chamber at 37 °C, the tissue was stained with 0.8% Ponceau 3R solution (Wako Pure Chemical Industries, Osaka) at room temperature for 6 min. The gelatinases in the tissue section were detected as negative staining areas with Ponceau 3R solution.

Extraction of SC Sheet

Human SC sheet stripped from sunburnt back skin of a volunteer was cut into pieces, which were added to a buffer containing 0.05 mol/L Tris-HCl and 0.15 mol/L NaCl and sonicated for 4 min. The solution was centrifuged at 10,000xg at 0 °C, and the supernatant was taken and used for gelatin zymography and ELISAs.

Gelatin Zymography

Ten microliters of the extract of SC sheet or enzyme solution was electrophoresed in a 10% sodium dodecyl sulfate-polyacrylamide gel containing 0.2% gelatin (Bio-Rad, Tokyo) at 100 V for 1.5 h. The gel was washed several times in a solution containing 2.5% Triton X-100, and transferred to a substrate buffer containing 5 mmol/L $CaCl_2$, 5 μmol/L $ZnCl_2$, and 0.05 mol/L Tris-HCl, pH 8.0, at 37 °C with shaking overnight. The gel was stained with 0.5% Coomassie Brilliant Blue R-250, which was dissolved in water containing 25% 2-propanol and 10% acetic acid, at room temperature for 30 min and destained with water containing 10% methanol and 10% acetic acid. Gelatinases in the skin extract solution were detected as unstained zones where gelatin had been degraded.

ELISAs

The amounts of MMP-2 and 9 were measured using ELISA kits (Amersham Life

Science, Buckinghamshire, UK) according to the manufacturer's protocol.

Gelatinase Coloring Test using FIZ-SN Film

The outer layer of SC was collected from the cheek or abdomen by gently pressing adhesive tape against the skin and then carefully removing the tape (tape stripping procedure). Samples were obtained from 100 healthy women (cheek, aged 45 - 55 years) and 2 men (abdomen, aged 40 and 48 years). The adhesive tape was put on a gelatin membrane containing colloidal silver with a thickness of 7 μm on polyester film (FIZ-SN film, Fuji Photo Film) and incubated at 37 °C for several days. FIZ-SN film has been reported to be suitable for detecting gelatinase because its colloidal silver reacts very sensitively and selectively with free SH groups of the gelatinase propeptide and its color changes from yellow to red or black [16].

UVB Irradiation

As a source of UVB, a Toshiba FL-20 SE fluorescent lamp (Toshiba Electric, Tokyo) was used. The lamp emits UV light mainly in the range of wavelength from 280 to 340 nm, with the maximum at 305 nm. The intensity of irradiation was measured by a UV-Radiometer (UVR-305/365D-II, Topcon, Tokyo). The abdomen was exposed to 1, 2, and 3 minimal erythema doses (MED) of UVB. The value of MED was determined prior to the irradiation.

Extraction of Rhizomes of *Curcuma longa* L. and Isolation of Gelatinase-Inhibitory Ingredients

Curcuma longa L. was extracted, and the major gelatinase inhibitor was isolated as follows. Dried rhizomes of *Curcuma longa* (5 kg) were extracted with ethanol at room temperature for 1 week. After removal of the solvent by evaporation, the residue (405 g)

was partitioned with hexane. The hexane-insoluble fraction (320 g, gelatinase-inhibitory part) was separated into a chloroform-acetone (95 : 5) fraction (144 g) and a methanol fraction (120 g) by silica gel column chromatography. The chloroform-acetone fraction (major inhibitory activity) was further purified and the main components were identified as curcuminoids. The structure of curcumin was confirmed by ^{1}H-, and ^{13}C-NMR, and its IC_{50} value for gelatinase inhibition was determined by means of a fluorimetric analysis procedure [17].

Results

1. Effects of Gelatinase on BM Structure in SE [18]

Abnormalities of BM, such as disruption or reduplication, can be observed in healthy human facial skin [5]. However, the mechanism of such damage is still unclear. We have investigated the factors that influence BM formation using a SE model. Although the BM component collagen IV was deposited at the dermal-epidermal junction, BM structure was discontinuous (Fig. 1A, C), and similar results were obtained by immunostaining of laminin 5 and collagen VII (data not shown). In spent media of SEs, gelatinases (MMP-2 and 9) which degrade collagen I, IV, and VII were detected (Fig. 1E). When an MMP-specific inhibitor (CGS27023A) was added to SE culture, it significantly accelerated the deposition of BM components and the BM continuity (Fig. 1B, D). Inhibition of degradation of type I collagen gel (Fig. 2), and the formation of BM structure were also confirmed. CGS27023A was more effective in promoting the deposition of BM components than other protease inhibitors examined (data not shown). These results suggest that gelatinase activity is closely associated with the degradation of BM structure.

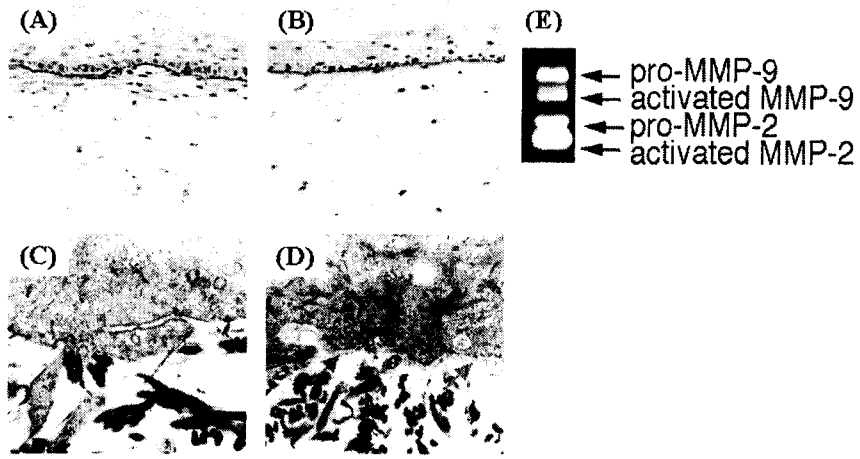


Fig. 1 Skin equivalent (SE) model. Matrix metalloproteinase (MMP) inhibitor, CGS27023A (10^{-5} mol/L) was added to the SE model (B and D). A and C: non-treated control. A and B were stained with an anti-human type IV collagen antibody. C and D are electron micrographs of A and B, respectively. CGS27023A enhanced deposition of type IV collagen at the junction between the epidermis and the dermis. The basal layer of epidermis was linearized by CGS27023A addition. Gelatinases (MMP-2, 9) are detected in spent media of SEs (E). Arrows show the basement membrane structure.

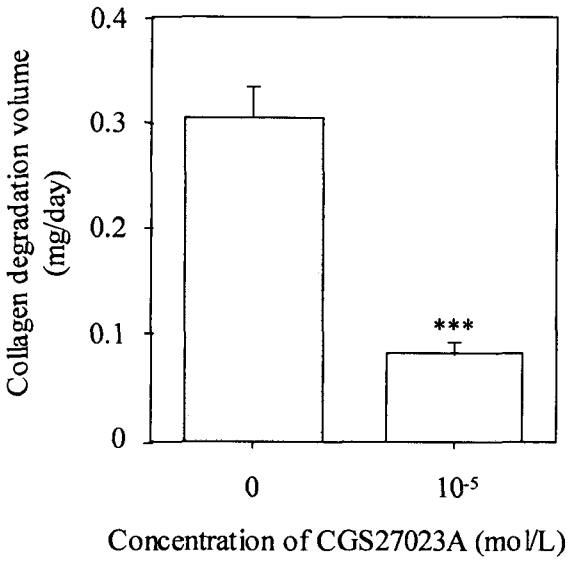


Fig. 2 Effect of CGS27023A (10^{-5} mol/L) on dermal collagen degradation in the skin equivalent (SE) model. Degradation of dermal collagen in the SE model was suppressed by CGS27023A. Each column with a bar indicates the mean value with standard deviation. ***: $p < 0.001$, $n = 3$, unpaired t-test.

2. Detection of Gelatinases in Healthy Human Skin

First, the presence and localization of gelatinase activity in healthy human facial skin (aged 59 years) were investigated by *in situ* gelatin zymography. Gelatinolytic activities were detected as a negative staining (white signal) area, representing the region in which gelatin had been digested, and were present throughout the epidermal layer of facial skin

(Fig. 3A, B), where BM integrity was severely impaired (Fig. 3C). However no gelatinolytic activity was found in the epidermis of non-irradiated skin (data not shown).

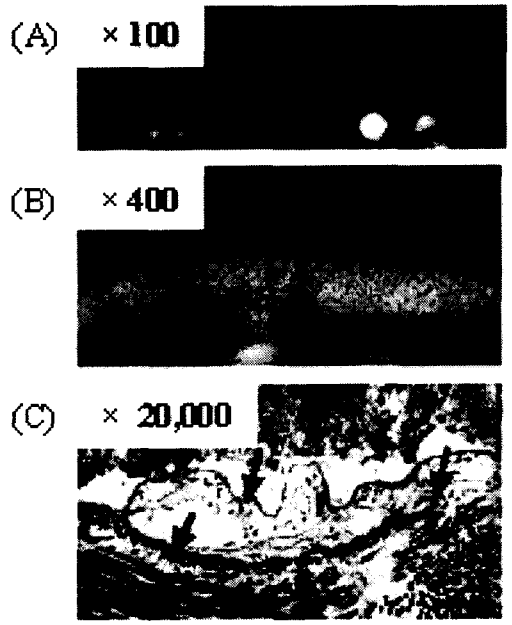


Fig . 3 Normal facial skin of a 59-year-old woman. (A) × 100, (B) × 400, (C) × 20,000, original magnification. (A and B) *In situ* zymography of the facial skin. Gelatinolytic activity was detected on the epidermis (Ponceau 3R staining). The broken line represents the boundary between the epidermis (the upper part) and the dermis (the lower part). (C) Ultrastructural study of the basement membrane (BM): same sample as in A and B. The BM is disrupted and reduplicated (arrows).

Next, the presence of gelatinase in SC was examined. The result of gelatin zymography showed that 92 and 72 kD gelatinolytic proteins existed in the extract of SC sheet collected from sunburnt back skin (Fig. 4A). They were suggested to be gelatinases, as the digestive activity disappeared upon addition of EDTA (Fig. 4B), and they were considered to be MMP-9 and 2, respectively, based on their molecular size [19]. ELISA confirmed the presence of gelatinases. The amount of MMP-9 was greater than that of MMP-2, in accordance with the result of zymography (Table 1). The immunostaining of SC of healthy facial skin was then examined. As shown in Fig. 5, MMP-9 was positively stained in SC from facial skin, while no staining was seen in SC from the non-exposed skin of the inside of the arms, or the facial skin stained with mouse IgG_1 (negative control). Staining was also negative in SC of other non-irradiated sites such as back or chest (data not shown). These results demonstrate that, in sun-exposed skin, gelatinase activity is always

upregulated around the basal layer of the epidermis and gelatinase is consequently present in the SC.

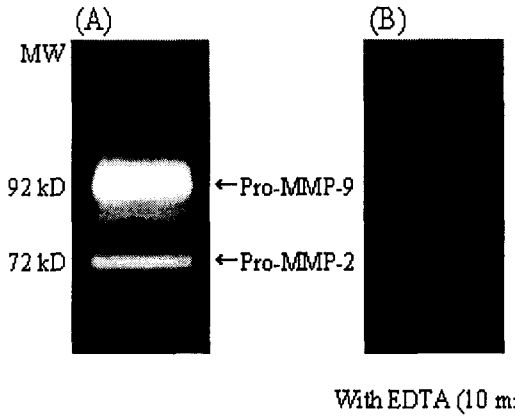


Fig. 4 Zymography of the extract of stratum corneum (SC) sheet taken from a volunteer. The gel on the right lane (B) had been incubated with 10 mmol/L EDTA. The bands at 92 kD and 72 kD were detected in the extract of SC from sunburnt back. These bands were thought to represent MMP-9 and MMP-2, based on their molecular size and the fact that their activity was inhibited by EDTA.

Table 1. Amounts of gelatinases B (MMP-9) and A (MMP-2) contained in stratum corneum (SC) extract evaluated by gelatinase-specific ELISA.

MMPs	ng / ml
Pro-MMP-9	40
Pro-MMP-2	16

SC sheet (40.4 mg) from sunburnt back was homogenized, and centrifuged, and the supernatant was used for ELISA.

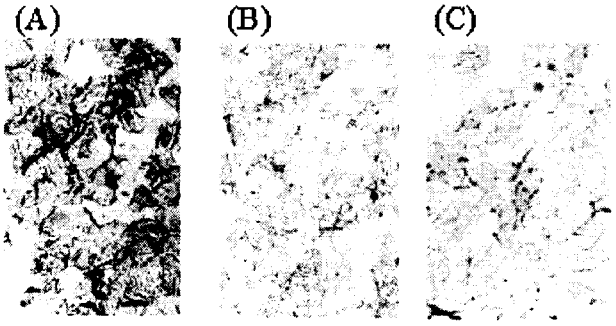


Fig. 5 Stratum corneum (SC) immunostained with an anti-human pro-gelatinase B monoclonal antibody. (A) Facial skin, (B) inside of the arm (non-exposed skin), and (C) the facial skin stained with mouse IgG_1 (the negative control). MMP-9 showed positive staining in SC from facial skin, while no staining was seen in SC from the inside of the arm or with the mouse IgG_1 (negative control).

3. Detection of SC Containing Gelatinase and Epidemiological Survey

3.1 Specific Detection of SC Containing Gelatinase by Using Gelatin Film Containing Colloidal Silver (FIZ-SN film)

In order to explore the behavior of gelatinase in epidermis non-invasively, a new detection method to identify gelatinase-containing SC was developed. We used a special film coated with a hard gelatin film containing colloidal silver, FIZ-SN film (Fig. 6). It has been reported that gelatinase activity coincided well with the sites colored by the colloidal silver when FIZ-SN film was used for *in situ* zymography of diseased tissues that contained gelatinase [16]. When a SC specimen was bonded onto FIZ-SN film with adhesive tape, the coloration was found within several days (Fig. 7A, C). As shown in Fig. 7, the pattern of color development on FIZ-SN film coincided well with the image obtained by staining with the pro-MMP-9 antibody, so the film was confirmed to detect gelatinase-containing SC as selectively and as sensitively as immunostaining. We further examined the detection of positive SC by FIZ-SN film in sunlight-unexposed skin which was irradiated with 1 - 3 MED to induce gelatinase in the basal layer of the epidermis. Positive SC was detected at about 18 days after the irradiation and disappeared by 28 days (Fig. 8). There was a linear relation between the amount of film-positive SC and the UVB-irradiation dose (data not shown). These results show that FIZ-SN film can sensitively and conveniently detect gelatinase-containing SC and that the gelatinase activity in epidermis can be detected non-invasively by coloration of the film by gelatinase in SC.

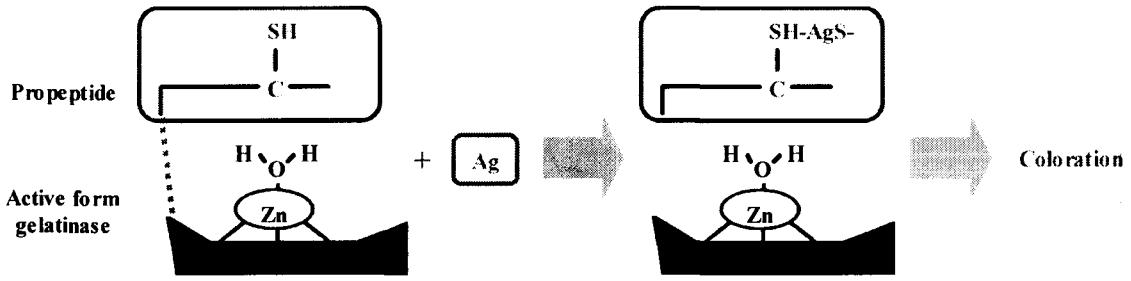


Fig. 6 Proposed mechanism of coloration of FIZ-SN film.

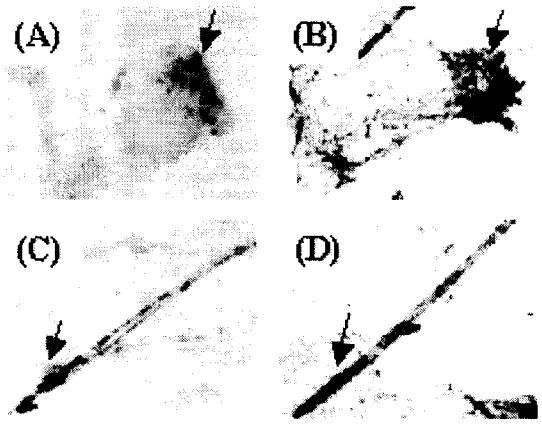


Fig. 7 Stratum corneum of facial skin was exposed to FIZ-SN film (A) and immunostained with an anti-human pro-gelatinase B antibody (B). Arrows indicate strongly stained or colored parts. The pattern of color development on FIZ-SN film coincided well with the image obtained by staining with pro-MMP-9 antibody. Photographs C and D show a hair shaft exposed on FIZ-SN film and immunostained for pro-gelatinase B, respectively. A clear stain was observed, in agreement with the reported finding that the lower hair shaft contains gelatinase.

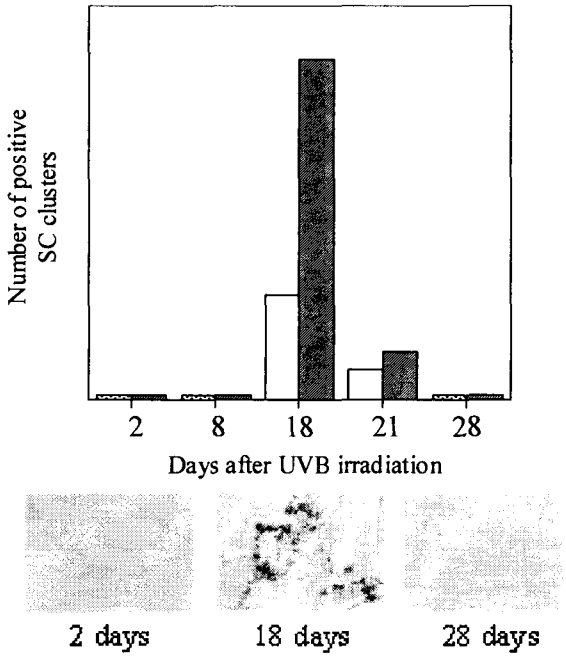


Fig. 8 Change in the number of colored stratum corneum clusters (SCC) on UVB-irradiated abdomen. The dotted (□) and hatched (▨) columns represent the number of positive SCC of 48- and 40-year-old men, respectively. The photographs show SCC of the 48-year-old man taken at 2, 18, and 28 days after UVB irradiation.

3-2 Survey of Gelatinase-Containing SC in Healthy Human Facial Skin

In order to explore the behavior of gelatinase in epidermis of healthy female human face, 100 SC samples were collected and the presence of gelatinase was investigated using the FIZ-SN film. As shown in Fig. 9 and Table 2, although the extent of the positive SC varied from subject to subject, positive SC was detected in 90% of the subjects tested. In skin unexposed to light, on the other hand, no positive SC was detected (Table 2). This

indicates that gelatinase is chronically upregulated in facial skin owing to exposure to UV in sunlight.

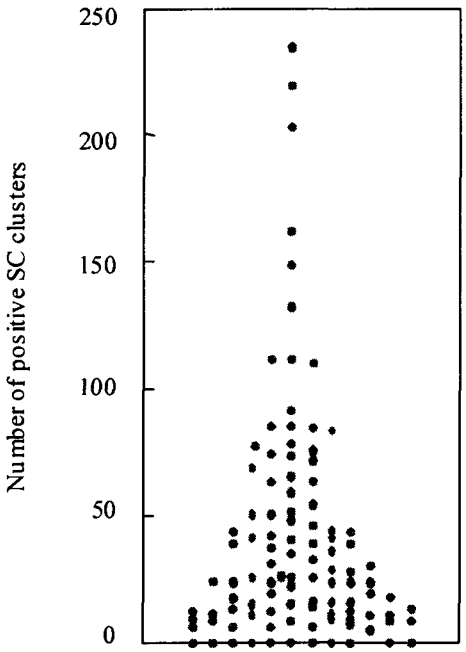


Fig. 9 Number of positive stratum corneum clusters on facial skin of 45 to 55-year-old women faces (n = 100).

Table 2. Results of epidemiological study of gelatinase in facial stratum corneum.

	Number of subjects	Number of subjects with positive stratum corneum	Positive rate (%)
Facial skin	100	90	90
Sunlight-unexposed skin	10	0	0

4. Natural Gelatinase Inhibitor, Turmeric Extract

4.1 The Inhibitory Effect of Turmeric Extract and Its Active Ingredient

The rootstock extract of *Curcuma longa* (ginger family) was proved to possess a potent inhibitory effect on gelatinase activity at the concentration of 0.002% or more (Fig. 10), and curcumin was identified as the main active ingredient of the extract, as shown in Fig. 11 (IC_{50} = 150 μmol/L). The effect of curcumin was further investigated using SE. The addition of curcumin to SE facilitated the deposition and improved the continuity of collagen IV (Fig. 12), and inhibited dermal collagen degradation (Fig. 13), having similar

effects to CGS27023A (Fig. 1). These results suggest that the main active ingredient of turmeric extract has gelatinase-inhibitory effect *in vitro*.

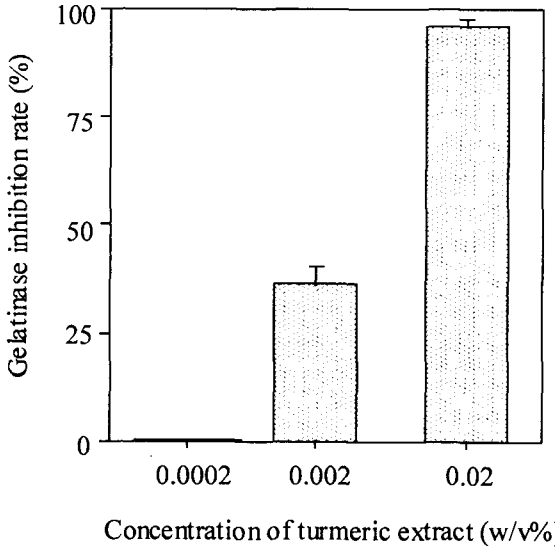
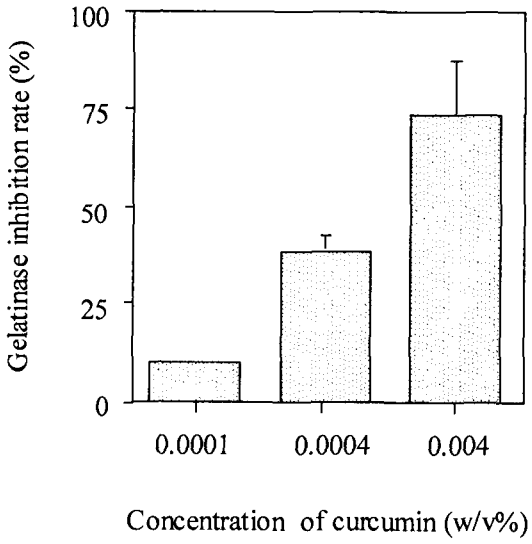


Fig. 10 Inhibitory effect of turmeric extract on gelatinase activity. Turmeric extract showed a strong inhibitory effect on gelatinase activity. Each column indicates the mean value with standard

(A)



(B)

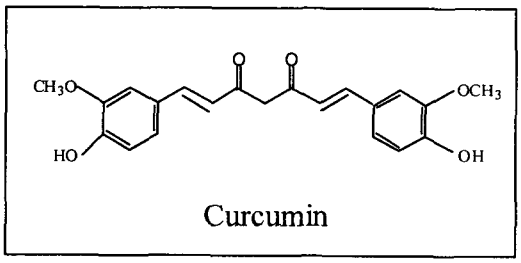


Fig. 11 Inhibitory effect of curcumin on gelatinase activity (A) and the chemical structure (B). The main active ingredient in turmeric extract was curcumin. The 50%-inhibitory concentration (IC_{50}) of curcumin was 1.5×10^{-4} mol/L. IC_{50} was measured by the method of Knight et al. [17]. Each column with a bar indicates the mean value with the standard deviation.

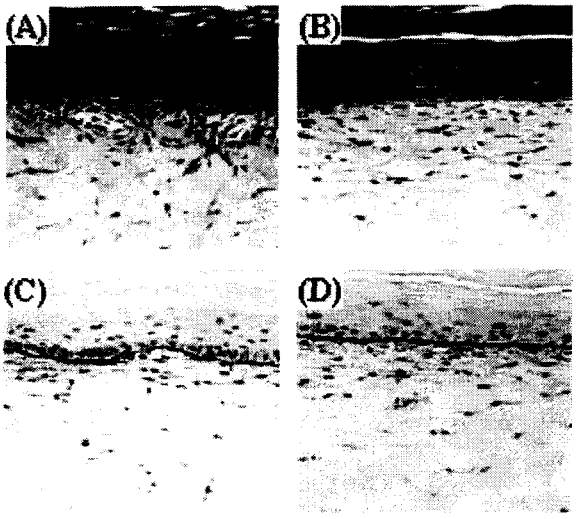


Fig. 12 Effect of curcumin on a skin equivalent model. Curcumin (10 ppm) was added to the skin equivalent model (B and D). A and C: non-treated control. Photographs A and B show the results of staining with hematoxylin and eosin; C and D were stained with an anti-human type IV collagen antibody. Curcumin enhanced deposition of type IV collagen at the junction between the epidermis and the dermis. The basal layer of epidermis was linearized by curcumin addition.

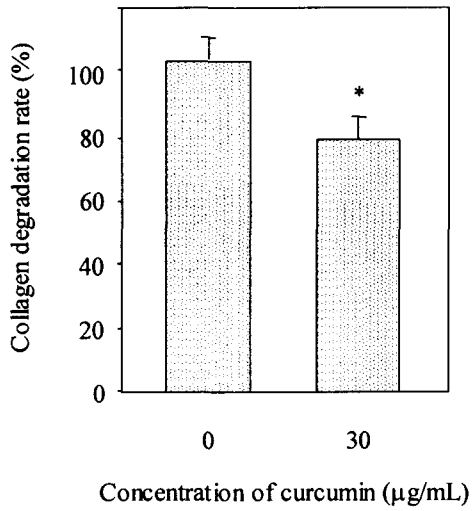


Fig. 13 Effect of curcumin on dermal collagen degradation in the skin equivalent model. Degradation of dermal collagen in the skin equivalent model was inhibited by curcumin. The data are the mean value of 3 measurements with the standard deviation.
*: $p < 0.05$, $n = 3$, unpaired t-test.

4.2 Study of Turmeric-Blended Cream as an Inhibitor of Gelatinase Activity in Human Facial Skin

The efficacy of turmeric extract in the inhibition of gelatinase activity in human facial skin was investigated. A cream containing turmeric extract was prepared, and the decrease of SC gelatinase activity after continuous external application of the cream for one month was used as an index of efficacy. Changes in the number of SC gelatinase clusters in volunteers and the results of statistical analysis are shown in Fig. 14. The turmeric extract-blended cream showed a marked inhibitory effect on gelatinase in facial epidermis. The number of colored SC clusters was decreased in eighteen subjects out of twenty-eight (efficacy rate: 64%). No adverse effect on the skin of any subject was observed during the

study period, indicating a high degree of safety of the cream used. These results suggest that the curcuma extract is a novel and effective component for cosmetics, which will be able to inhibit effectively the chronically activated, aging-associated epidermal gelatinase in sunlight-exposed skin.

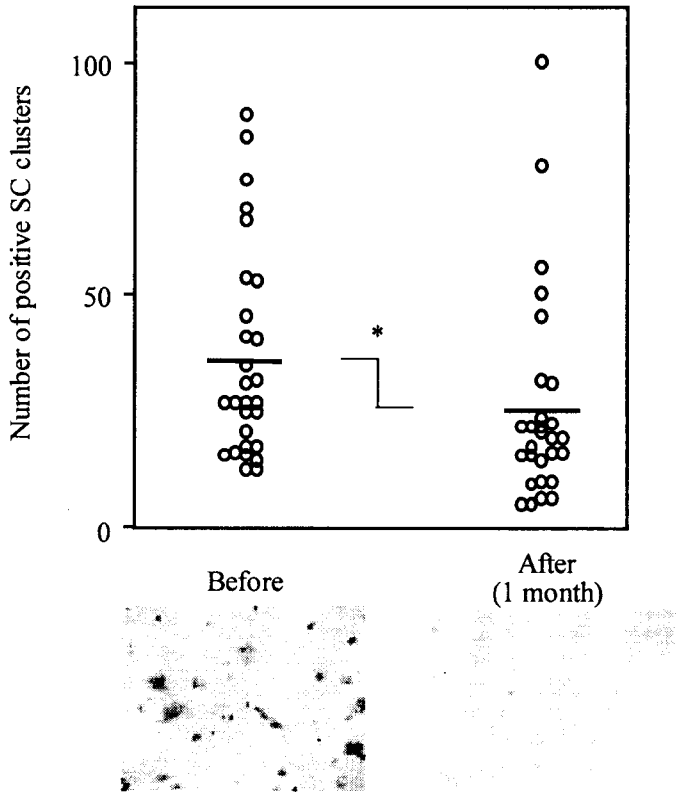


Fig. 14 Number of positive stratum corneum clusters (SCC) before and after treatment with turmeric extract-blended cream for one month. Turmeric extract-blended cream produced a statistically significant decrease in the number of gelatinase-containing SCC on the face. The horizontal bars indicate the mean value of data before and after the treatment. The photographs represent typical example of before and after application. *: $p < 0.05$, $n = 28$, paired t-test.

Discussion

There have been a few studies on the behavior of MMPs in normal skin tissue. Ashcroft et al. showed that intrinsic aging accelerated MMP-2 production [20], and Ohnishi reported that MMP-1 and 2 were activated in facial skin with actinic elastosis [21]. However, because of the ethical unacceptability of surgically invasive sampling, little is known about the behavior of MMPs in healthy female human skin, which is cosmetically an important target. In this study, we have conducted for the first time a survey on the behavior of gelatinase in facial epidermis of healthy female volunteers by using gelatin *in situ* zymography (FIZ-SN film) in combination with a non-invasive SC sampling method (Figs. 3 and 7). The use of FIZ-SN film enabled us to detect gelatinase with high sensitivity, comparable to that of antibody staining, and to visualize specifically gelatinase-containing SC without any staining process (Fig. 7). This easy and convenient technique made it possible to evaluate large numbers of SC specimens.

The behavior of gelatinase was investigated through three methods, *in situ* gelatin zymography, immunological staining of SC using a gelatinase-B-specific antibody, and etiologic investigation of gelatinase-containing SC in 100 volunteers. The results showed that gelatinase activity exists around the basal layer of the epidermis in healthy human facial skin (Fig. 3), and the activity can be detected in SC (Figs. 4-7, and Table 1). Gelatinase activity was detected in facial SC of more than 90% of the volunteers (Fig. 9 and Table 2). In non-irradiated skin, on the other hand, gelatinase activity was undetectable in both the epidermis and the SC (Table 2). These results suggest that gelatinase is chronically upregulated by sunlight in the epidermis of healthy human facial skin.

Gelatinase can degrade BM constituents such as collagen IV or collagen VII [22]. Our previous study showed that BM was markedly reduplicated and disrupted in

sun-exposed facial skin, and the degree of damage increased with advancing age [5]. In the present study, we found that gelatinase impairs the BM structure in a SE model. Further, gelatinase activity was detected around the basal layer of epidermis, where BM integrity was severely impaired. These results suggest that gelatinase is at least partly responsible for the change of BM structure in sun-exposed human facial skin.

UV is strongly associated with the expression of gelatinase in skin [7, 8]. Fisher *et al.* found that MMP-1, 2, 3, and 9 were activated by low-dose UVB in non-irradiated skin [7]. It has also been reported that low-dose UVB-irradiation induced gelatinase around the basal layer of the epidermis in an *in vivo* photoaging model, and repeated exposure caused BM damage and formation of wrinkles [9], while UVA induced no wrinkle formation [23]. This report is consistent with our observation that UVB exposure of non-irradiated skin produced a dose-dependent increase of gelatinase activity in SC after the turnover time of SC, whereas 20 J of UVA irradiation produced no change in the amount of gelatinase-containing SC. Therefore, it is suggested that gelatinase present in human facial SC might be evoked by the constant exposure to low doses of UVB in sunlight during daily life.

The possible utility of plant extracts having gelatinase-inhibitory effects that might be used as safe cosmetic ingredients has been widely explored, since it has been reported that application of a gelatinase-inhibitory substance suppressed UVB-induced BM impairment and prevented the development of wrinkles in an *in vivo* photoaging model [9]. We discovered a strong gelatinase-inhibitory effect in the rootstock extract of *Curcuma longa* L. (turmeric extract). The extract was expected to be safe and pharmacologically active, because it has long been used as a traditional Chinese medicine or Jamu medicine, and also as a food ingredient for curry powder [24]. Curcumin, a major component of turmeric extract, has anti-inflammatory, anti-oxidative and anti-tumor activities [25-28], as well as

metal-chelating ability [29, 30]. As most MMP-inhibitory substances act through chelation of zinc, which is a component of the enzyme active center, the mechanism of gelatinase-inhibitory activity of curcumin is likely to be metal chelation [31].

Gelatinase-inhibitory effects of curcumin or turmeric extract were investigated using a SE model and human facial skin, respectively. Curcumin treatment accelerated collagen IV deposition and improved the continuity around the D-E junction in the SE model. Topical application of turmeric extract-blended cream acted to decrease the gelatinase activity in SC in facial skin, suggesting that the cream significantly suppressed the activity of gelatinase chronically generated by UVB in epidermis.

Taken together, these results suggest that turmeric extract effectively suppresses upregulated gelatinase activity, which accelerates BM damage and collagen degradation in sun-irradiated facial skin, and it is thus a promising cosmetic ingredient to ameliorate photoaging and to maintain well-conditioned skin.

Conclusion

We found gelatinase that degrades BM and is involved in photoaging is chronically activated in healthy female facial skin using a novel method to detect gelatinase in SC non-invasively. Application of turmeric extract-blended cream decreased gelatinase in SC effectively, suggesting that turmeric is an effective ingredient to prevent skin from photoaging by suppressing chlonically upregulated gelatinase activity by UV and to improve skin condition.

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