UV Irradiation Induces the Expression of Gelatinases in Human Skin

In vivo

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UV irradiation results in marked changes in skin connective tissue, such as degeneration of collagen, and abnormal elastosis. The mechanism of connective tissue damage by UV has not been clarified in detail. In the present study the mechanism of actinic damage was studied by assaying gelatinases, 72-kDa (MMP-2) and 92-kDa (MMP-9), from suction blister fluids induced on patients who had received either UVB or PUVA treatments. The results indicate that both UVB and PUVA increase the levels of gelatinases in human skin. By in situ hybridization, it was also possible to show that UV irradiation induced increased levels of gelatinase mRNAs in fibroblasts. Furthermore, in samples from severe actinic damage, gelatinase mRNAs were abundantly present, suggesting that gelatinases may contribute to photodamage. Key words: UVB; PUVA; photodamage; 72-kDa gelatinase; 92-kDa gelatinase.

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Aging of human skin represents a complex situation, where numerous intrinsic and extrinsic factors contribute to the changes. One of these factors is UV radiation, which causes premature aging and actinic damage (1–3). Histologically, actinically damaged human skin shows deposition of disorganized elastotic material and basophilic degeneration of collagen. These changes are particularly pronounced in the papillary dermis in the early stages of the damage, but in advanced cases similar changes are noted in the reticular dermis (2). Biochemical studies have revealed alterations in collagen, which is the major component of the dermis. In particular, a reduced concentration of hydroxyproline, as an index of collagen, has been found (2, 4). The degeneration of collagen found in actinic damage could result from increased degradation by proteolytic enzymes. Indeed, recent studies have shown that UV radiation stimulates collagenase in cultured fibroblasts (5–7). Furthermore, UV radiation in humans in vivo has shown to increase collagenase mRNA in fibroblasts, suggesting that UV radiation can increase collagenase which could then degrade collagen (3).

Collagenases are a family of several enzymes including interstitial collagenase (MMP-1), 72-kDa (MMP-2) and 92-kDa (MMP-9) gelatinases, and stromelysin (MMP-3) (8, 9). Gelatinases have the ability to degrade gelatin, basement membrane collagen and some other collagen types such as V, VII, and X (8–11). The effects of UV radiation on the expression of gelatinases has not been studied. For this reason we have now measured gelatinases from patients who have been treated with UVB or PUVA. The presence of gelatinases was also studied by in situ hybridization from UV-treated skin as well as from severe actinic damage. The results indicate that UV radiation increases the expression of gelatinases, which could contribute to photodamage.

MATERIALS AND METHODS

Patients
Blister fluid samples were obtained from 26 psoriatic patients. All of the patients were males. Seven had not received UV radiation (mean age 46, range 28–68); 11 psoriatic patients had received topical PUVA therapy (mean age 45, range 27–65) and 8 had received UVB therapy (mean age 40, range 26–59) for long time periods (12). Two patients with other skin diseases had received UVB therapy and they were included in the UVB group. Suction blisters were induced on the healthy-looking abdominal skin and the blister fluid was collected with a manitous syringe and kept at −20°C until assayed (13).

The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Oulu, and the work was carried out according to the provisions of the Declaration of Helsinki.

Assay of gelatinase by zymography
Seventy-two-kDa and 92-kDa molecular weight gelatinases were assayed from suction blister fluids from patients. Our previous studies have shown that detectable levels of gelatinases are present in suction blisters (14).

The method of Heasen & Dowdle was used for zymography, except that the substrate was gelatin-labelled with 2-methoxy-2, 4-diphenyl-3(2H)-furinone (Fluka, Ronkonkoma, N.Y.) according to the method of O'Grady et al. (15). The advantage of this method is that lysis can be monitored visually, or by photographic inspection under long-wave ultraviolet light, and the capacity of the proteins in the samples to bind Coomassie brilliant blue does not affect the pattern of lysis. Prior to electrophoresis, the samples for zymography were preincubated with 1% SDS and electrophoretic sample buffer, and preincubated molecular weight standard proteins were reduced with 5% 2-mercaptoethanol. Zymography was performed in 1.5 mm 10% polyacrylamide slab gels containing 2 mg/ml gelatin. The gels were photographed under long-wave ultraviolet illumination. Cleavage rates were estimated by determining the rate of disappearance of the gelatin by densitometric scanning of the negatives of the photographed gels using a computer densitometer model 300A (Molecular Dynamics). Five μl blister fluid samples were usually used for the assay, which has been shown to be linear from 1 to 10 μl volume of blister fluid (14).

Skin specimens
Skin biopsies were taken under local anaesthesis from non-lesional skin of 11 subjects with psoriasis (2 had been treated mostly with UVB, 7 with PUVA for a long time, and 2 had not received any treatment). All the samples were obtained from abdominal sun-protected skin, which clinically did not show any significant actinic damage. In addition, samples were obtained during routine operations from facial skin with actinic elastosis of 2 subjects and from sun-protected skin of 5 controls without psoriasis. The samples were fixed in formalin and mounted in paraffin. Five-μm sections were cut for in situ hybridization and routine hematoxyline eosin sections. Elastic was stained with Verhoef – van Gieson.

In situ hybridization
A detailed description of the preparation of paraffin sections for in situ hybridization has been given previously (16). For hybridization with
The levels of gelatinases in non-treated, PUVA- or UBV-treated patients expressed as densitometric units. The means of levels are shown by short bars.

Statistical analysis

For statistical analysis the Mann-Whitney U-test was used.

RESULTS

Demonstration of gelatinases in suction blister fluids and effects of PUVA and UBV treatments

Gelatinases were assayed by the zymography method, which allows both the type 72-kDa and type 92-kDa enzymes of gelatinase to be measured simultaneously. The levels of 72-kDa and 92-kDa gelatinases were lower in non-treated patients, compared to PUVA and UBV-treated patients (Fig. 1). The mean level of 72-kDa gelatinase was in non-treated patients 128 DU (median 114), in PUVA-treated patients 224 DU (median 235, p = 0.027, compared to non-treated patients) and in UBV-treated patients 176 DU (median 183). The mean level of 92-kDa gelatinase was in non-treated patients 60 DU (median 37), in PUVA-treated patients 122 DU (median 119) and in UBV-treated patients 125 DU (median 122, p = 0.043, compared to non-treated patients).

In situ hybridization

The in situ hybridization for the presence of 72-kDa and 92-kDa gelatinase mRNAs revealed abundant signals, especially for 72-kDa gelatinase in fibroblasts of UV-treated skin (Fig. 2). Signals were also found in endothelial cells (see also ref. 14). The signals in PUVA- and UBV-treated subjects were in general more abundant than in non-treated subjects or controls. Only occasionally could gelatinase mRNAs be seen in skin samples obtained from non-treated skin or controls.

Seventy-two-kDa gelatinase mRNA was also demonstrated abundantly in fibroblasts of skin biopsies taken from subjects.
Fig. 2. In situ hybridization for the presence of 72-kDa gelatinase mRNA in a skin specimen (from the abdominal skin of a 41-year-old male who had been treated with UVB and PUVA for 4 years) shows specific grains in dermal fibroblasts (arrows). Endothelial cells (arrowheads) of a dermal capillary (c) also contain hybridization signals (magnification ×400) (A). The negative control exhibits the background reaction only (B).

with severe photodamage (Figs. 3, 4). In other specimens obtained from sun-protected skin, such an accumulation of 72-kDa gelatinase mRNA could not be seen.

DISCUSSION

The results indicate that therapeutic UVB and PUVA treatments can increase the expression of gelatinases in human skin. Especially interesting was the finding of a high signal for 72-kDa gelatinase mRNA in samples obtained from subjects with severe solar elastosis. Thus it is possible that actinic damage by UV radiation is partially due to gelatinases. Gelatinases have a broad spectrum of action on various components of connective tissue. They can degrade not only basement membrane collagen and gelatin but also elastin. It is possible that the fragmentation of elastin noted in some PUVA-treated patients could be due to increased levels of gelatinases (2).

How UV radiation induces gelatinases is still unknown. As regards interstitial collagenase, studies have emphasized the role of various interleukins, which can stimulate interstitial collagenase (19). In vivo and in vitro studies have shown that UV radiation increases the levels of interleukins (20–22).

Another possibility is that UVA radiation, which can penetrate into the dermal site of the skin, could directly stimulate

Fig. 3. In situ hybridization for the presence of 72-kDa type IV collagenase of a skin specimen (from the tip of the nose of a 66-year-old subject) with solar elastosis shows numerous specific grains in a fibroblast near the epidermis in the centre of the field (arrow) (magnification ×400).

Fig. 4. In situ hybridization for the presence of 72-kDa type IV collagenase of a skin specimen (from the cheek of an 86-year-old subject) with a heavy solar elastosis reveals specific grains in fibroblasts in the mid-dermal area in the vicinity of a hair follicle (arrows) (magnification ×400).

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collagenases through stimulation of protein kinase C-tumour promoting phorbol ester cascade (23, 24). In the case of UVB, which is mostly absorbed into the epidermis, the mechanism could be through numerous cytokines as discussed above.

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