Ultraviolet Irradiation Increases Matrix Metalloproteinase-8 Protein in Human Skin In Vivo

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Humans express three distinct collagenases, MMP-1, MMP-8, and MMP-13, that initiate degradation of fibrillar type I collagen. We have previously reported that ultraviolet irradiation causes increased expression of MMP-1, but not MMP-13, in keratinocytes and fibroblasts in human skin in vivo. We report here that ultraviolet irradiation increases expression of MMP-8 in human skin in vivo. Western analysis revealed that levels of the full-length, 85 kDa proenzyme form of MMP-8 increased significantly within 8 h post ultraviolet irradiation (2 minimal erythema doses). Increased full-length MMP-8 protein was associated with infiltration into the skin of neutrophils, which are the major cell type that expresses MMP-8. Immunofluorescence revealed coexpression of MMP-8 and neutrophil elastase, a marker for neutrophils. Immunohistology demonstrated MMP-8 expression in neutrophils in the papillary dermis between 4 and 8 h post ultraviolet irradiation, and in the epidermis at 24 h post irradiation. MMP-8 mRNA expression was not detected in nonirradiated or ultraviolet-irradiated human skin, indicating that increased MMP-8 following ultraviolet irradiation resulted from preexisting MMP-8 protein in infiltrating neutrophils. Pretreatment of skin with the glucocorticoid clobetasol, but not all-trans retinoic acid, significantly blocked ultraviolet-induced increases in MMP-8 protein levels, and neutrophil infiltration. In contrast, all-trans retinoic acid and clobetasol were equally effective in blocking ultraviolet induction of MMP-1 and degradation of collagen in human skin in vivo. Taken together, these data demonstrate that ultraviolet irradiation increases MMP-8 protein, which exists predominantly in a latent form within neutrophils, in human skin in vivo. Although ultraviolet irradiation induces both MMP-1 and MMP-8, ultraviolet-induced collagen degradation is initiated primarily by MMP-1, with little, if any, contribution by MMP-8. Key words: collagen/glucocorticoid/neutrophil collagenase/retinoid acid. J Invest Dermatol 117:219–226, 2001

Photoaging, caused by repeated exposure to solar ultraviolet (UV) irradiation, results in both clinical and histologic changes in human skin (Havlik et al., 1999). Characteristic clinical features of photodamaged skin include both fine and coarse wrinkling, mottled pigmentation, dryness, and loss of skin tone (Gilchrest and Yaar, 1992; Scharffetter-Kochanek, 1997). Histologic and ultrastructural studies have revealed that the major alterations in photoaged skin are localized in the connective tissue (Scharffetter-Kochanek, 1997), which is composed predominantly of collagen, elastin, proteoglycans, and fibronectin. As collagen fibrils and elastin are responsible for the strength and resilience of skin (Uitto, 1986), their degeneration with photoaging causes skin to become less youthful in appearance. Biochemical, quantitative, and qualitative changes have been reported in the dermal extracellular proteins elastin (Braverman and Fonferko, 1982; Uitto, 1986), interstitial collagen (Trautinger et al., 1989; Schwartz et al., 1991), and glycosaminoglycans (Sams and Smith, 1961; Smith et al., 1962) in photoaged skin. Histologically, connective tissue damage induced by UV irradiation is primarily manifested as the disorganization of collagen fibrils (Bernstein et al., 1996) that constitute the bulk (90% dry weight) of skin connective tissue, and accumulation of abnormal, amorphous, elastin-containing material (Lavker, 1995).

The matrix metalloproteinases (MMPs) are a family of enzymes responsible for degrading connective tissue (Murphy et al., 1990; Woessner, 1994). They are structurally related endopeptidases that mediate degradation of different macromolecular components of the extracellular matrix and the basement membrane (Matrisian, 1990; Woessner, 1991). The human family of MMPs is composed of at least 16 members, which can be classified into four different subfamilies; the collagenases, the gelatinases, the stromelysins, and the membrane MMPs (Shingleton et al., 1996; Kahari and Saarialho-Kere, 1997). In humans, there are three distinct collagenases: MMP-1 (collagenase 1 or interstitial collagenase), MMP-8 (neutrophil collagenase or collagenase 2), and MMP-13 (collagenase 3) (Shingleton et al., 1996; Vincenti et al., 1996). MMP-1 is expressed by keratinocytes and fibroblasts and functions in normal collagen turnover and matrix remodeling during wound healing. MMP-8 is expressed predominantly by neutrophils and released during inflammatory processes (Hasty et al., 1990; Devarajan et al., 1991). MMP-13 is expressed in various epithelial cancers but, unlike MMP-1, it is not expressed by either keratinocytes or...
fibroblasts during wound repair. MMP-13, however, is expressed in fibroblasts in chronic cutaneous ulcers (Ravani et al., 1999). There is significant homology between MMP-8 and MMP-1: MMP-8 exhibits 57% identity to the protein sequence for MMP-1 (Hasty et al., 1990). MMP-8 cleaves type I collagen faster than type III collagen, whereas MMP-1 shows greater selectivity for type III collagen relative to type I collagen (Hasty et al., 1987; Hirose et al., 1993; Knauper et al., 1997). Both MMP-1 and MMP-8 are synthesized as latent proenzymes that require proteolytic processing to become catalytically active. Whereas MMP-1 is synthesized and released from cells into the extracellular matrix, however, MMP-8 is synthesized and stored in specific granules in neutrophil leukocytes (Hasty et al., 1986; Knauper et al., 1990; Mallya et al., 1990). MMP-8 activity is therefore regulated by factors such as surface-bound ligands (IgG or complement components) that release it through degranulation (Chatham et al., 1990). Once released and activated through proteolytic or oxidative mechanisms (Weis, 1989), MMP-8 plays a major role in the connective tissue turnover that accompanies inflammatory processes (Weiss et al., 1985; Knauper et al., 1990; Claesson et al., 1996; Okamoto et al., 1997).

UV irradiation induces MMP-1, but not MMP-13, in human skin in vivo. In addition, UV irradiation induces 92 kDa gelatinase (MMP-9) and stromelysin-1 (MMP-3) in human skin in vivo (Fisher et al., 1996). Together, these three MMPs can fully degrade skin collagen (Matrisian and Hogan, 1990; Birkedal-Hansen et al., 1993). Initially, MMP-1 cleaves the triple-helical collagen molecule into three-quarter and one-quarter length fragments. Collagen is then further degraded into smaller fragments by MMP-3 and MMP-9 (Hasty et al., 1990; Shingleton et al., 1996; Kahari and Saarialho-Kere, 1997). MMPs are likely to be the primary mediators of connective tissue damage in skin exposed to UV irradiation (Fisher et al., 1997).

To better understand the mechanism of skin collagen breakdown associated with the photoaging process, we have investigated whether UV irradiation alters MMP-8 expression in human skin in vivo. We have also examined the effects of different classes of compounds, all-trans retinoic acid and the glucocorticoid clobetasol, which inhibit UV induction of MMP-1, on expression of MMP-8 in human skin in vivo.

MATERIALS AND METHODS

Procurement of human skin biopsies Sun-protected buttocskin of adult Caucasian subjects, who were without current or prior skin disease, was pretreated with 0.1% all-trans retinoic acid, 0.05% clobetasol propionate, or its vehicle (70% ethanol, 30% propylene glycol, and 0.05% butylated hydroxytoluene) for 24 h under occlusion prior to UV exposure. Sun-protected buttocskin of subjects was exposed to twice the minimal erythema dose (2 MED) of UV. Two UV sources were utilized: (i) four FS6T12 ERE-VHO UVB tubes filtered with Kodacel to remove wavelengths below 290 nm (Fisher et al., 1997); and (ii) a 450 W xenon arc solar simulator filtered with a Schott WG320 filter to remove wavelengths below 290 nm, coupled to a liquid light guide. The spectral output of these two sources was determined with an OLS754 spectroradiometer (Optronic Laboratories, Orlando, FL). The UV spectrum (290–400 nm) for UVB was composed of 0.3% UVC, 65.1% UVB, 2.1% UV-A2, and 10.2% UVA1. The UV spectrum for the xenon arc lamp was 0.00006% UVC, 6.6% UVB, 16.5% UV-A2, and 76.8% UVA1. Light output was monitored with an IL 4443 phototherapy radiometer and a SED240/UVB/UV photodetector (International Light, Newbury, MA).

For studies with multiple time points, tissue was obtained from each subject at each time point. Replicate 4 mm or 6 mm punch biopsies of skin were obtained from irradiated and adjacent nonirradiated sites. Skin samples were obtained at the times indicated following UV irradiation. For studies with multiple time points, tissue was obtained from each subject at each time point. Replicate 4 mm or 6 mm punch biopsies of skin were obtained from irradiated and adjacent nonirradiated sites. Skin samples for enzyme-linked immunosorbent assay (ELISA), Western analysis, and reverse transcriptase polymerase chain reaction (RT-PCR) were snap-frozen in liquid nitrogen and stored at −70°C. Skin samples for immunohistology were oriented in OCT embedding compound (Miles Laboratories, Elkhart, IN) prior to snap freezing. The University of Michigan Institutional Review Board approved all procedures involving human subjects, and all subjects provided written informed consent.
mouse monoclonal IgG₁ antibody (Dako). Appropriately diluted mouse IgG₁ was used as control.

**ELISA analysis for MMP-8 and MMP-1 proteins**  MMP-8 and MMP-1 proteins were assayed with the Biotrak capture ELISA (Amersham Pharmacia, Arlington Heights, IL). For determining MMP-8 protein levels, skin samples were homogenized by vigorous vortexing with 2.5 mm glass beads (Biospec, Bartlesville, OK) in 20 mM Tris (pH 7.6), 5 mM CaCl₂, containing protease inhibitor mixture. The homogenate was centrifuged at 10,000 × g for 10 min and the supernatant was collected for analyses. MMP-1 protein was assayed from supernatant obtained from skin biopsies that had been incubated in Dulbecco’s modified Eagle’s medium (Life Technologies, Rockville, MD) for 8 h at 37°C. Protein content was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA), using bovine serum albumin as standard.

**Western analysis of MMP-8 protein**  MMP-8 protein levels in supernatants from skin homogenates, prepared as described above for ELISA analysis, were determined by Western blot analysis, as described previously (Fisher et al., 1996). Equal amounts of protein (100 μg per lane) were analyzed for each treatment group. Immunoreactive MMP-8 protein was detected by enhanced chemiluminescence (Amersham Pharmacia) using a monoclonal antibody against human MMP-8 that detects both the full-length latent (85 kDa) and active (64 kDa) forms (Matsuki et al., 1996). Immunoblots were visualized and quantified by STORM PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**RNA isolation and RT-PCR**  Total RNA from human skin, primary cultured human keratinocytes, and fibroblasts was purified by guanidine-thiocyanate extraction (Stratagene, La Jolla, CA). MMP-8 mRNA expression was assessed by RT-PCR analysis. Briefly, cDNA was prepared using a constant amount of total cellular RNA (1 μg), reverse transcriptase, and random oligo primers. The cDNA was amplified in the presence of specific primers for human MMP-8 (5'-AGC TGT CAG AGG CTG AGG TAG AAA G and 5'-CCT GAA AGC ATA GTT GGG ATA CAT). Amplification conditions were denaturation for 40 s at 94°C, annealing for 60 s at 55°C, and extension for 90 s at 72°C for 33 cycles. RT-PCR amplification of the mRNA for the ribosomal protein 36B4 served as internal control. Reaction products were subjected to electrophoresis on 1.5% agarose gel, visualized with Vistra Green (Molecular Probes, Eugene, OR), and quantified by STORM PhosphorImager (Molecular Dynamics).

**Measurement of degraded collagen**  Partially degraded collagen in the dermis of nonirradiated and UV-irradiated human skin was quantified by modifications of the procedure described by Bank et al. (1997). This assay is based on the ability of α-chymotrypsin to extensively hydrolyze partially degraded, but not intact, native insoluble fibrillar collagen. The soluble proteolytic fragments derived from collagen are quantified by hydroxyproline content. Full-thickness skin samples were homogenized in 20 mM Tris HCl (pH 7.3) containing 1 mM iodoacetamide, 1 mM ethylenediamine tetraacetic acid, and pepstatin A (10 μg per ml) and centrifuged at 50,000 rpm for 40 min. Pellets were washed three times with 5 mM Tris (pH 7.3) and centrifuged at 10,000 × g for 5 min. α-Chymotrypsin was added to the pellets (75 μg in 200 μl in 5 mM Tris) and incubated at 37°C for 8 h. Reaction mixtures were centrifuged at 10,000 × g for 10 min, and proteolytic fragments released into supernatants were hydrolyzed with 6 N HCl at 110°C for 24 h. Hydrolysates were dried under vacuum, dissolved in distilled water, and redried under vacuum to remove traces of HCl. Finally, samples were dissolved in 0.1 M borate buffer (pH 9.5) and derivatized with o-phthalaldehyde and 9-fluorenylmethyl chloroformate according to the procedure of Bank et al. (1997). Derivatized hydroxyproline, derived from collagen, was quantified by high performance liquid chromatography using an analytical C-18 Econosphere column. Isocratic elution of hydroxyproline was performed with 20 mM citric acid containing 5 mM tetrathylammonium chloride (pH 2.85) and 38% acetonitrile, at a flow rate of 1 ml per min. Derivatized hydroxyproline was measured by fluorescence at 330 nm (excitation wavelength 254 nm), using a Hitachi (Model 1080) fluorescence detector.

**Statistics**  Time course data, and data from comparisons of vehicle-treated skin to skin pretreated with all-nano retinolic acid or clobetasol, were analyzed with paired t tests. All p-values are two-tailed, and differences were considered significant for p < 0.05.
RESULTS

UV irradiation induces MMP-8 protein, but not MMP-8 mRNA, levels in human skin in vivo

MMP-8 protein was minimally detectable by Western blot analysis in extracts from nonirradiated human skin (Fig 1A). MMP-8 protein levels were increased approximately 4-fold within 8 h and remained elevated for 24 h after UV irradiation (Fig 1A). By 48 h post-UV, MMP-8 protein levels returned to near-baseline levels. Western analyses detected the full-length 85 kDa latent form of MMP-8. The processed 64 kDa active form of MMP-8 was not detectable.

MMP-8 protein levels in human skin extracts were quantified by ELISA. In nonirradiated human skin, MMP-8 protein levels were less than 0.5 ng per mg tissue homogenate protein. MMP-8 protein levels were increased to 5.4 ng per mg protein within 8 h following UV irradiation (Fig 1B). MMP-8 protein levels remained elevated for 24 h, consistent with the western analysis described above (Fig 1A).

We next performed RT-PCR to determine whether UV increased MMP-8 mRNA. MMP-8 mRNA was not detected in either nonirradiated or UV-irradiated human skin in vivo. Additionally, no MMP-8 mRNA was detect by RT-PCR in nonirradiated or UV-irradiated cultured human keratinocytes or dermal fibroblasts. Control MMP-8 cDNA was reproducibly amplified, indicating that lack of detection of MMP-8 mRNA in skin was not due to failure of the PCR amplification. In addition,
36B4 mRNA was amplified in all skin samples examined (N = 10, data not shown), indicating that RNA preparations were suitable for the RT-PCR analysis.

**UV irradiation induces infiltration of neutrophils expressing MMP-8** The above data suggest that preformed MMP-8 protein is brought into the skin following UV irradiation. To examine this issue, we utilized immunohistology to localize MMP-8 protein in human skin.

Immunohistology revealed increased expression of MMP-8 protein in cells in the dermis within 8 h post-UV (Fig 2A, B). Cells expressing MMP-8 protein were prominently localized near blood vessels in the dermis. MMP-8-positive cells were detected in both papillary dermis and epidermis by 24 h post-UV (Fig 2C). At 48–72 h post-UV, MMP-8-positive cells were minimally detectable (data not shown), as was observed in non-UV-irradiated skin.

The above data demonstrate that UV irradiation induces influx into skin of cells expressing MMP-8 protein. Neutrophils express MMP-8 and are known to infiltrate skin following UV irradiation (Hawk et al., 1988; Strickland et al., 1997). To determine whether these MMP-8-expressing cells were neutrophils, we performed immunohistology for neutrophil elastase, a marker for neutrophils (Lammers et al., 1986), in UV-irradiated and nonirradiated skin. Not surprisingly, an influx of neutrophil-elastase-positive cells was detected in UV-irradiated skin (Fig 2D–F). Localization of neutrophils within the skin at 8 h (Fig 2E) and 24 h (Fig 2F) after UV irradiation was similar to that for the MMP-8-expressing cells.

We utilized immunofluorescence to confirm that the MMP-8-expressing cells were in fact neutrophils in UV-irradiated human skin. Sections from human skin obtained 24 h post UV irradiation were double-stained for MMP-8 and the neutrophil marker, neutrophil elastase (Fig 3). MMP-8 exclusively colocalized with neutrophil-elastase-positive cells (i.e., neutrophils). Interestingly, not all neutrophils expressed detectable levels of MMP-8.

The UV source used for the above studies emitted predominantly UVB wavelengths (65.1% of total emitted energy from UVB). Solar UV is composed primarily of UV wavelengths. We therefore determined the ability of solar-simulated UV, which closely resembles Solar UV is composed primarily of UV wavelengths. We therefore determined the ability of solar-simulated UV, which closely resembles

**Glucocorticoid, but not all-trans retinoic acid, inhibits UV induction of MMP-8 protein in human skin in vivo** Previous studies have shown that pretreatment with all-trans retinoic acid (Fish et al., 1996) or glucocorticoid (Fisher et al., 1996) inhibits UV induction of MMP-1 in keratinocytes and fibroblasts in human skin in vivo. We therefore examined the effect of these two compounds on UV induction of MMP-8 protein in human skin in vivo. We therefore examined the effect of these two compounds on UV induction of MMP-8 protein in human skin in vivo. Subjects were treated with 0.1% all-trans retinoic acid or 0.05% clobetasol for 24 h prior to UV irradiation of skin, and skin samples were obtained 24 h post-UV. Pretreatment of human skin with the glucocorticoid clobetasol significantly inhibited UV induction of MMP-8 protein, as determined by both Western blot analysis (Fig 5A) and ELISA (Fig 5B). In contrast, all-trans retinoic acid did not significantly inhibit UV induction of MMP-8 protein in human skin (Fig 5A, B).

Immunohistology revealed that skin pretreated with clobetasol prior to UV exposure exhibited significantly fewer MMP-8-expressing cells (Fig 6D), and significantly fewer infiltrating neutrophils (Fig 6H), relative to skin pretreated with vehicle (Fig 6B, F). Pretreatment of skin with all-trans retinoic acid, however, had no significant inhibitory effect on either MMP-8 expression (Fig 6C) or neutrophil infiltration into skin (Fig 6G).

**UV-induced collagen breakdown in human skin in vivo is associated with MMP-1, but not MMP-8** We have previously demonstrated that UV irradiation induces MMP-1 mRNA, protein, and activity in human skin in vivo (Fisher et al., 1996, 1997). Both MMP-1 and MMP-8 can initiate cleavage of insoluble fibrillar type I and type III collagen in human skin. We next determined the relative contributions of MMP-1 and MMP-8 to UV-induced collagen breakdown in human skin in vivo. Insoluble fibrillar collagen that is cleaved by MMP-1 or MMP-8 is susceptible to further degradation by proteases other than MMPs. We utilized this differential sensitivity to cleavage by protease other than MMPs to determine the levels of partially degraded insoluble collagen in UV-irradiated human skin in vivo. UV irradiation induced a 3-fold increase in degraded collagen, 24 h following a single 2 MED UV exposure, compared to nonirradiated skin (Fig 7A). As expected, the levels of both MMP-1 and MMP-8 proteins were significantly elevated in the same UV-irradiated skin that contained increased degradation of insoluble collagen.

**Figure 5. Pretreatment of skin with glucocorticoid, but not all-trans retinoic acid, inhibits UV induction of MMP-8 protein in human skin in vivo.** Human skin was pretreated with vehicle (VEH), 0.1% all-trans retinoic acid (tRA), or 0.05% clobetasol (CLO) for 24 h under occlusion. Skin was irradiated with UV (2 MED) and skin samples were obtained 24 h post-UV. (A) MMP-8 protein levels in skin were measured by Western analysis. Data represent means ± SEM, and are expressed as fold increase in MMP-8 protein levels relative to levels in nonirradiated skin. Inset shows a representative Western blot. N = 8, *p < 0.05 vs nonirradiated control. (B) MMP-8 protein levels in skin samples were assayed by ELISA. Data represent means ± SEM, and are expressed as nanograms MMP-8 protein per milligram extract protein. *p < 0.05 vs nonirradiated control. N = 12.
Degraded collagen (Fig 7A). UV induction of MMP-1 was substantially inhibited (approximately 70%) by pretreatment of skin with either all-trans retinoic acid or clobetasol (Fig 7B). In contrast, UV induction of MMP-8 was not inhibited by all-trans retinoic acid but was substantially inhibited (80%) by clobetasol. Importantly, pretreatment of skin with either all-trans retinoic acid or clobetasol inhibited UV-induced collagen degradation to a similar extent (60%, Fig 7B). In other words, clobetasol pretreatment, which inhibited UV induction of both MMP-1 and MMP-8, was no more effective in inhibiting UV-induced collagen degradation than all-trans retinoic acid, which inhibited induction of MMP-1 but did not inhibit induction of MMP-8. These data indicate that UV-induced collagen degradation in human skin in vivo is primarily dependent on MMP-1, but not MMP-8.

DISCUSSION

We have demonstrated that UV irradiation induces MMP-8 protein in human skin in vivo using three independent methods—Western blot analysis, ELISA, and immunohistochemistry. We observed significant increases in MMP-8 protein within 8 h post-UV, which were maintained for 24 h. Double staining immunofluorescence revealed that MMP-8 was expressed in neutrophils identified as neutrophil-elastase-positive cells in UV-exposed human skin. Our own data and those in previous studies (Hawk et al, 1988; Strickland et al, 1997) demonstrate that peak infiltration of neutrophils occurs between 8 and 24 h after UV exposure.

Recent studies have shown that, in addition to its expression in neutrophils, MMP-8 protein is also found in human articular chondrocytes, rheumatoid synovial fibroblasts, and endothelial cells (Chubinskaya et al, 1996; Cole et al, 1996; Hanemaaijer et al, 1997). We found that neither normal cultured human keratinocytes nor skin fibroblasts, however, expressed MMP-8 mRNA following UV exposure. These results are consistent with our inability to detect MMP-8 mRNA in untreated and UV-irradiated human skin. We conclude that UV irradiation of human skin does not induce MMP-8 gene expression in resident skin cells, but rather causes recruitment of neutrophils that contain preformed MMP-8 protein into skin.

Degradation of type I collagen is initiated by one of three mammalian collagenases (MMP-1, MMP-8, and MMP-13). We have previously demonstrated that UV irradiation induces interstitial collagenase (MMP-1) in the epidermis and dermis, and causes degradation of type I collagen in human skin in vivo (Fisher et al, 1997). We now show that UV irradiation also induces neutrophil collagenase (MMP-8) in human skin in vivo. Further, MMP-8 was induced to similar levels following 2 MED exposure to either a UVB-enriched source or a source that emits predominantly UVA and has an emission spectrum that closely resembles natural sunlight.

MMP-8 is synthesized as a latent glycosylated proenzyme with an apparent molecular weight of 85 kDa (Hasty et al, 1986; Mookhtiar and Van Wart, 1990) that is stored in specific neutrophilic granules (Manardi et al, 1991). Following secretion, a 79–81 amino acid N-terminal fragment is cleaved to produce a 64 kDa active enzyme (Murphy et al, 1977; Knauper et al, 1990, 1993). Western analysis revealed increased levels of the full-length 85 kDa MMP-8 in human skin following UV irradiation (Fig 1). The 64 kDa active form of MMP-8 was not detected, however (Fig 1). In addition, immunohistochemistry and immunofluorescence revealed that MMP-8 was localized within neutrophils in UV-irradiated human skin. No secreted extracellular MMP-8 staining was detectable. These data suggest that MMP-8 in UV-irradiated skin is retained within neutrophils in an inactive state. Conversely, MMP-8 was not detectable in at least 50% of infiltrating neutrophils, raising the possibility that MMP-8 was released upon entry or during migration of neutrophils into the skin. It is likely that diffusion of low levels of released MMP-8 throughout the skin would not be detectable by immunohistochemistry. In addition, once activated, MMP-8 is rapidly broken down (Knauper et al, 1993), thereby hampering its detection by Western analysis. Although we attempted to directly measure MMP-8 activity in UV-irradiated human skin, the sensitivity of the assay was not sufficient based on the levels of MMP-8 protein present in UV-irradiated skin extracts as determined by ELISA.

UV induction of MMP-8 differs in two important respects from UV induction of MMP-1, MMP-3, and MMP-9 in human skin. First, UV induction of MMP-1, MMP-3, and MMP-9 is
expression of inflammatory cytokines and adhesion molecules, which are necessary for neutrophil infiltration into skin (Barnes and Adcock, 1993). We utilized the differential effect of all-trans retinoic acid on UV induction of MMP-8 and MMP-1 to study the contribution of these two collagenses to UV-induced collagen fragmentation in vivo. Clofetrol and all-trans retinoic acid pretreatment reduced UV-induced collagen degradation to similar extents (60%). As all-trans retinoic acid inhibited UV induction of MMP-1 but not MMP-8, whereas clofetrol inhibited UV induction of both MMP-1 and MMP-8, we conclude that MMP-8 does not significantly contribute to UV-induced collagen degradation. Thus, MMP-1 is the primary initiator of collagen breakdown in UV-irradiated human skin in vivo.

Taken together, our data indicate that UV irradiation elevates MMP-8 protein levels by recruiting neutrophils containing preformed MMP-8 in human skin in vivo. Induction of MMP-8 protein by UV can be inhibited by pretreating skin with clofetrol, a potent anti-inflammatory agent, but not with all-trans retinoic acid. The majority of MMP-8 in UV-exposed skin is localized within neutrophils in an inactive latent form, and does not appear to significantly contribute to UV-induced collagen degradation.

Figure 7. MMP-1, but not MMP-8, is associated with UV-induced collagen breakdown in human skin in vivo. (A) Skin samples were obtained from nonirradiated skin (open bars) or UV-irradiated skin 24 h following exposure (2 MED) (closed bars). MMP-1 and MMP-8 protein levels were measured by ELISA. UV-induced degradation of insoluble collagen was measured in matched skin samples by susceptibility to proteolytic degradation by α-chymotrypsin, as described in Materials and Methods. Data presented are means ± SEM, N = 16, *p < 0.01. (B) Human skin was pretreated with vehicle, all-trans retinoic acid (open bar), or clofetrol (closed bar) for 48 h prior to UV exposure (2 MED, UVB source). MMP-1 protein, MMP-8 protein, and collagen degradation were determined as described above in matched skin samples obtained 24 h after UV irradiation. Data are presented as percent inhibition of UV-induced MMP-1, MMP-8, and collagen degradation in clofetrol- or all-trans retinoic acid-pretreated skin relative to vehicle-pretreated skin. Data are means ± SEM, N = 8, *p < 0.002 vs vehicle + UV; †p < 0.002 vs all-trans retinoic acid + UV.

**REFERENCES**


