Upregulation of MMP12 and Its Activity by UVA1 in Human Skin: Potential Implications for Photoaging

Angela Tewari[1], Katarzyna Grys[2], Jutta Kollet[3], Robert Sarkany[1] and Antony R. Young[1]

UVA1 constitutes around 75% of the terrestrial UV radiation, and most of the output of artificial tanning sources. However, the molecular effects of UVA1 in human skin *in vivo* are surprisingly poorly understood. We have examined time-dependent whole-genome expression, along with mRNA and protein changes in the skin after one minimal erythema dose of spectrally pure UVA1 (50 J cm^{-2}) and 300 nm UVB (30 mJ cm^{-2}). After 24 hours, the genes induced to the greatest extent were those involved in extracellular matrix remodeling with both UVA1 ($P = 5.5e-7$) and UVB ($P = 2.9e-22$). UVA1 and UVB caused different effects on matrix metalloproteinase (MMP) expression: UVB induced MMP1, MMP3, and MMP10 mRNA at 24 hours to a much greater extent than UVA1. MMP12 induction by UVA1 at 6 hours is marked and much greater than that by UVB. We have found that MMP12 mRNA induction by UVA1 resulted in expression of MMP12 protein, which is functional as an elastase. This induction of elastase activity did not occur with UVB. We hypothesize that the UVA1 induction of MMP12 mediates some of its photoaging effects, particularly by contributing to elastin degeneration in late solar elastosis. MMP12 is a good marker of UVA1 exposure.

Journal of Investigative Dermatology (2014) **134,** 2598–2609; doi:10.1038/jid.2014.173; published online 8 May 2014

INTRODUCTION

Terrestrial solar UV radiation (UVR) comprises <5% UVB (~295–315 nm) and >95% UVA (315–400 nm), the majority (~75%) of which is UVA1 (340–400 nm). UVA1 also makes up most (~80%) of the spectral output of sunbeds and is used at high doses as a specialist form of phototherapy (Kerr *et al.*, 2012). However, the acute effects of UVA1 *in vivo*, including its effects on gene expression, are much less well understood than those of UVB (Enk *et al.*, 2004; Enk *et al.*, 2006). As it is now clear that UVA1 is biologically active and mutagenic in human skin *in vivo* (Mouret *et al.*, 2006; Tewari *et al.*, 2012), it is clinically important to understand the biological effects of UVA1 in the skin. Previous studies on the effects of UVA1 on whole-genome expression *in vivo* have been limited and have primarily focused on understanding mechanisms of pigmentation (Choi *et al.*, 2010).

The hallmarks of long-term exposure to solar UVR are photoaging (Yaar and Gilchrest, 2007) and photocarcinogenesis (Sage *et al.*, 1996). Photoaging is characterized by the induction of extracellular matrix-degrading proteolytic enzymes (matrix metalloproteinases, MMPs) without a parallel induction of inhibitors of proteolysis (tissue inhibitor of metalloproteinases). The resulting pathological remodeling process involves the degradation of collagen and the accumulation of abnormal elastin in the superficial dermis, resulting in the characteristic changes of solar elastosis (Chen *et al.*, 1986; Uitto, 2008). Previous studies with UVR sources rich in UVB showed induction of MMP1, MMP3, and MMP9 mRNA (Brenneisen *et al.*, 1996; Fisher *et al.*, 1996; Fisher *et al.*, 1997) in human skin *in vivo*. UVA sources have also been reported to induce MMP1 expression in fibroblasts (Scharffetter *et al.*, 1991; Herrmann *et al.*, 1993). There are few data on the effects of spectrally pure UVA1 on MMPs in human skin *in vivo* (Wang *et al.*, 2013).

MMP activity is required both in normal physiological processes such as wound healing and angiogenesis (Chakraborti *et al.*, 2003) and in the pathological tissue destruction that occurs in chronic wounds, dermal photoaging, bullous skin disease, cancer invasion, and metastasis (Kerkela and Saarialho-Kere, 2003). The mechanisms by which UVR induces MMPs are poorly understood. Some studies have reported that this occurs via the generation of reactive oxygen species (Scharffetter-Kochanek *et al.*, 1993; Fisher *et al.*, 2009), whereas others have suggested that DNA is a major chromophore and that MMPs are triggered by the formation of cyclobutane pyrimidine dimers (CPDs) (Dong *et al.*, 2008). MMPs may also be induced by mediators such as tumor necrosis factor α (Steenport *et al.*, 2009), which is also readily induced in the skin by UVR, probably via the induction of

[1]*King's College London, King's College London School of Medicine, Division of Genetics and Molecular Medicine, St John's Institute of Dermatology, London, UK;* [2]*St John's Institute of Dermatology, NIHR GSTFT/KCL Biomedical Research Centre, Guy's Hospital, London, UK and* [3]*Bioinformatics, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany*

Correspondence: Angela Tewari, King's College London, King's College London School of Medicine, Division of Genetics and Molecular Medicine, St John's Institute of Dermatology, London, UK. E-mail: angela.tewari@kcl.ac.uk

Abbreviations: MMP1, matrix metalloproteinase 1; MMP12, matrix metalloproteinase 12; UVA1, ultraviolet A1 (340–400 nm)

Received 9 April 2013; revised 22 February 2014; accepted 11 March 2014; accepted article preview online 8 April 2014; published online 8 May 2014

CPDs (Walker and Young, 2007) and IL6 (Wlaschek *et al.*, 1994).

It is widely considered that UVA has a larger role than UVB in photoaging, both because of the deeper penetration of UVA into the dermis (Bruls *et al.*, 1984) and because of the sensitivity of fibroblasts to UVA-induced MMPs (Scharffetter *et al.*, 1991; Herrmann *et al.*, 1993). There are inherent problems with previous studies that relate to spectral purity of the source of UVR. UVB sources often also emit a considerable amount of UVA and it is not possible to attribute an effect to UVB without knowledge of the action spectrum of the end point under investigation. Even minor contamination of a nominal UVA source with very small amounts of UVB may give misleading results because <1% UVB contamination can be responsible for most of a given effect, e.g., DNA damage (Woollons *et al.*, 1999). Also, UVA2 (315–340 nm) and UVA1 are biologically and mechanistically different. Thus, we have concentrated our studies on the biological effects of spectrally pure UVB (300 nm) and UVA1. In a recent work, we reported that solar UVB exposure was probably the most important factor in MMP1 induction from an environmentally relevant perspective (Tewari *et al.*, 2012). In this study, we compare the effects *in vivo* of erythemally equivalent and biologically relevant doses of spectrally pure UVA1 and UVB on whole genome expression, mRNA, and protein and enzyme activity of the most significantly enriched pathway at 24 hours (extracellular matrix remodeling). We have used this approach because erythema is the widely used end point in clinical and experimental photodermatology. We chose 300 nm because it is in the region of the peaks of the action spectra both for erythema and for CPD induction in human skin *in vivo* (Young *et al.*, 1998) and its photobiological effects are likely to be mechanistically different from UVA1. Furthermore, erythemal exposure, quantified by the standard erythema dose, is increasingly used as a measure of UVR exposure in clinical and epidemiological studies.

RESULTS

Extracellular matrix remodeling genes are induced to a greater extent than other pathways, both by UVA1 and by UVB 24 hours after exposure.

We used Genego Metacore v7 on our microarray data to identify upregulated pathways at 6 and 24 hours using the pooled intensities ($n=9$ for UVA1 and $n=5$ for UVB) of upregulated genes compared with each individual's nonirradiated control ($P<0.05$, fold change $\geqslant 2$). At 6 hours, the most significantly enriched pathway was inflammation through Th17 signaling for erythemally equivalent doses of UVA1 ($P=1.16e-6$) and UVB ($P=2.1e-4$). At 24 hours, the most significantly enriched pathway was extracellular matrix remodeling for UVA1 ($P=5.5e-7$) and UVB ($P=2.9e-22$). Many other groups of genes were induced to lesser extents but this paper will focus on the MMPs, given their striking degree of induction.

Whole-skin microarray analysis at 6 (Figure 1a) and 24 hours (Figure 1b) after exposure indicated that genes encoding MMP1, MMP3, MMP9, MMP10, and MMP12 are induced predominantly at 24 hours. MMP1, MMP3, and MMP10 (10–550-fold, $P\leqslant 0.05$, adjusted $P\leqslant 0.3$) were induced to a greater extent by UVB, whereas UVA1 induced a 15-fold increase in MMP12 gene expression at 6 hours ($P\leqslant 0.05$, adjusted $P\leqslant 0.3$) that increased to around 30-fold at 24 hours ($P\leqslant 0.05$, adjusted $P\leqslant 0.3$).

Key UVA1 and UVB mRNA expression differences

Specific genes assessed by reverse transcription quantitative PCR (qPCR) at 6 and 24 hours are shown in Figure 1c–h. At 24 hours, UVB was more effective than UVA1 at inducing MMP1 ($P=0.0062$, UVB/UVA1 fold difference 13.2) (Figure 1c), MMP3 ($P=0.0016$, UVB/UVA1 fold difference 38.7) (Figure 1d), and MMP10 ($P=0.028$, UVB/UVA1 fold difference 27.1) (Figure 1e). Both UVA1 and UVB induce MMP9 mRNA to an equal extent (at 6 and 24 hours post exposure (Figure 1f); $P=0.33$ at 6 hours, $P=0.12$ at 24 hours). UVA1 was more effective than UVB at inducing MMP12 mRNA at 6 ($P=0.02$, UVA1/UVB fold difference 19.2) and 24 hours ($P=0.22$, UVA1/UVB fold difference 8.26). However, the large interindividual variation at 24 hours means that the difference is not significant (Figure 1g). UVA1 did not induce MMP3 or MMP10.

UVR-induced MMP1 protein and its activity in the epidermis

Typical MMP1 and MMP12 protein staining (red fluorescence) and enzyme activity (*in situ* zymography shown by green fluorescence) are shown in Figure 2a and b, respectively. UVB and UVA1 induce MMP1 protein (at 10 and 24 hours (Figure 2c)) to a similar extent. Enzyme activity is preferentially induced by UVB at 24 hours compared with UVA1 ($P=0.031$) (Figure 2d). DQ collagen type I is a substrate for MMP1, MMP2, MMP3, MMP9, and MMP13 (Yan and Blomme, 2003). Figure 3a shows that MMP2 protein was not induced by either spectrum at 24 hours. However, as shown in Figure 3b, UVB induced an increase in MMP9 protein at 24 hours.

UVA1 preferentially induces MMP12

MMP12 protein is predominantly formed by UVA1 at 24 hours ($P=0.04$) (Figure 2e). A lesser degree of induction of MMP12 protein is seen with UVB at 10 and 24 hours, despite the absence of MMP12 mRNA induction by UVB at 6 and 24 hours. There is significantly ($P=0.027$) more MMP12 activity at 10 hours after UVA1 exposure than after UVB (Figure 2f).

Location of MMP1 and MMP12 proteins within the epidermis

Figure 2g and h show that MMP1 protein is mainly induced in the upper epidermis (upper versus lower epidermis UVB $P=0.005$ and UVA1 $P=0.01$), whereas MMP12 is equally induced throughout the epidermis (Figure 2i and j) (upper vs. lower epidermis UVB $P=0.20$, UVA1 $P=0.42$).

UVR-induced MMPs and their activity in the dermis

Expression of MMP1 and MMP12 proteins in the dermis are higher with UVA1 than UVB (Figure 4a and c), but there are no spectral differences in the enzyme activities (Figure 4b and d). Overall, both MMP protein and enzyme activity are higher in the epidermis than in the dermis. Figure 5a shows

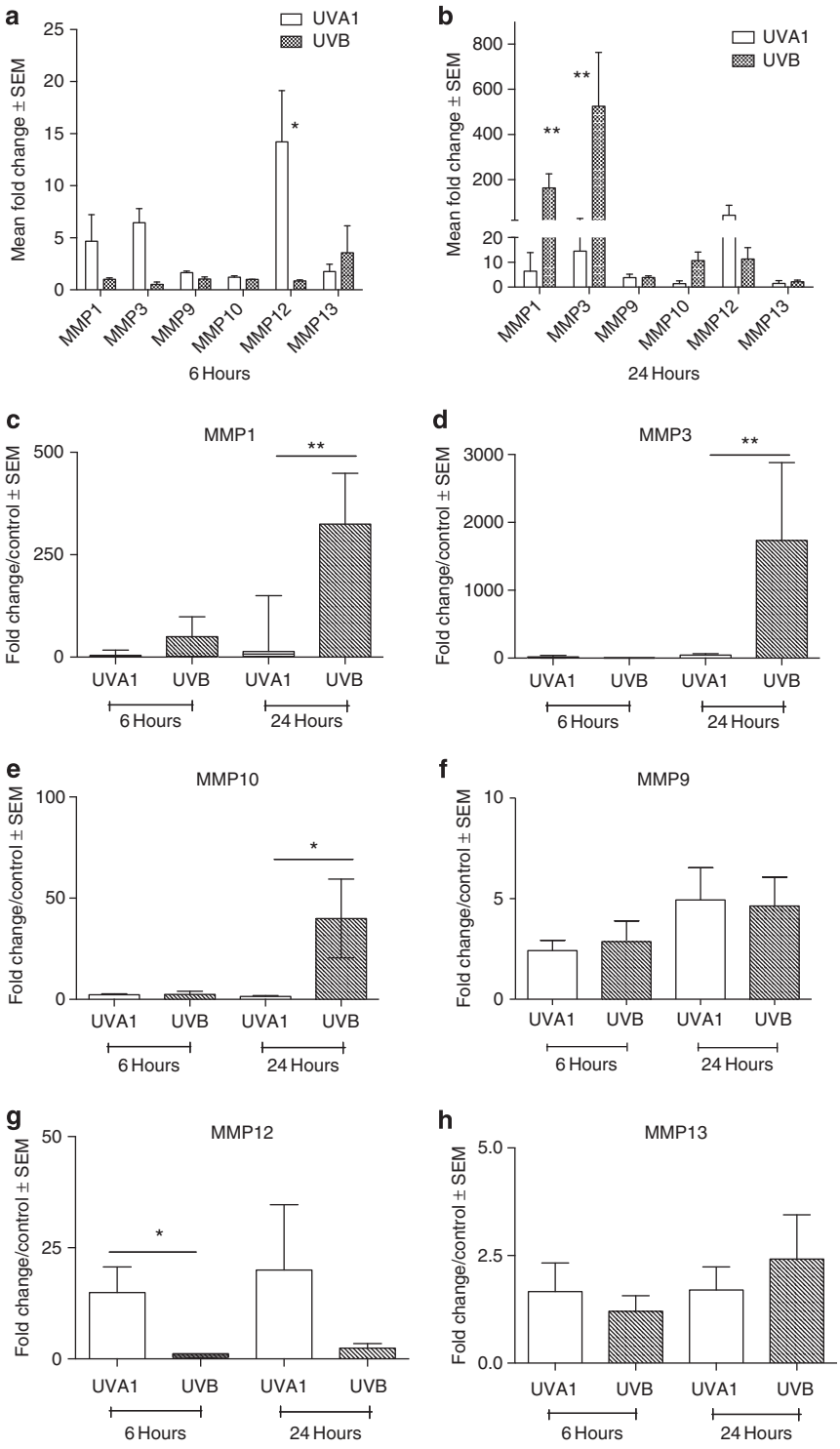

Figure 1. Key UVA1 and UVB gene expression differences in extracellular matrix remodeling: RNA changes. Nine skin type I/II participants for UVA1 and five skin type I/II participants for UVB, RNA extracted and converted to cRNA, and hybridized to Agilent 4 × 44 K microarray chips. Normalized gene expression ratios (log_2) compared with each individual's nonirradiated control at (**a**) 6 hours and (**b**) 24 hours were used for calculations. The individual values were plotted ($P \leqslant 0.05$, adjusted $P \leqslant 0.3$). Validation with reverse transcriptase (RT) quantitative (PCR) qPCR (ΔΔCT method) using Taqman probes and GAPDH housekeeping gene to produce a relative fold change: (**c**) MMP1, (**d**) MMP3, (**e**) MMP10, (**f**) MMP9, (**g**) MMP12, (**h**) MMP13. *$P \leqslant 0.05$, **$P \leqslant 0.01$. Microarray and qPCR were performed on samples from all nine volunteers.

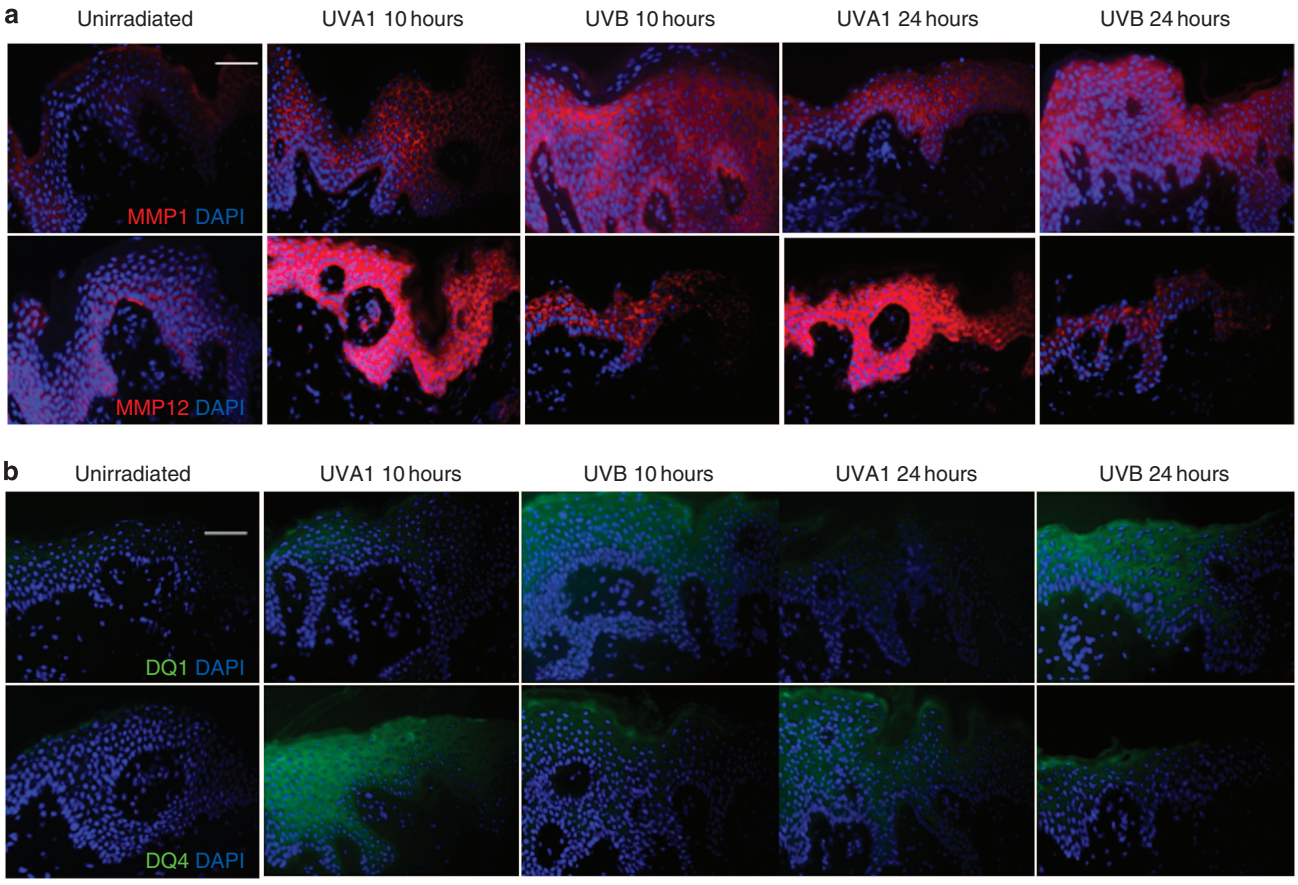

Figure 2. UVA1 induces more epidermal matrix metalloproteinase (MMP)12 protein (immunofluorescence) and enzyme activity (*in situ* zymography) than UVB. MMP1 and MMP12 protein (antibodies from Abcam) immunofluorescence in one representative individual detected by (**a**) alexafluor 555 (red fluorescence) for 30 mJ cm^{-2} UVB (~1 minimal erythema dose (MED)) at 10 and 24 hours and 50 J cm^{-2} (~1MED) of UVA1 at 10 and 24 hours and (**b**) DQ collagen type I (DQ1), substrate for MMP1, MMP2, and MMP9, and DQ collagen type IV (DQ4), substrate for MMP2, MMP7, MMP9, MMP12, and MMP25, were used to detect enzyme activity via fluorescein (green fluorescence), quantification of (**c**) epidermal MMP1, (**d**) epidermal DQ1, (**e**) epidermal MMP12, and (**f**) epidermal DQ4. Distribution of MMP1 and MMP12 within the epidermis, (**g**) UVB-induced MMP1, (**h**) UVA1-induced MMP1, (**i**) UVB-induced MMP12, and (**j**) UVA1-induced MMP12. *$P \leqslant 0.05$, **$P \leqslant 0.01$; $n = 3$. Scale bar = 50 μm.

that UVA1, but not UVB, induces elastin breakdown in the epidermal region.

Macrophages are a potential source of UVA1-induced MMP12

Figure 5b shows significant depletion of dermal macrophages (CD68$^+$) 24 hours after UVB exposure. In contrast, UVA1 had no effect on dermal macrophage numbers ($P = 0.21$) compared with nonirradiated controls.

DISCUSSION

We have compared UVR-induced MMPs using spectrally pure UVB (300 nm) and UVA1 on the same individuals, which is likely to reduce the impact of interpersonal variation. Our UVA1 doses are physiologically relevant; 50 J cm^{-2} would be a typical dose received from a 2.5 hour exposure to the tropical Australian sun (19 °S) (Bernhard *et al.*, 1997) and higher doses are regularly given for treatment of sclerosing skin conditions (Kerr *et al.*, 2012).

Baseline expression of MMPs is usually low in human tissue, including skin. Our gene array data (supported by qPCR) show that erythemally equivalent doses of UVB and UVA1 induce many MMPs. There was no increase in tissue inhibitor of metalloproteinases 1–4 with either spectrum assessed by microarray (data not shown). For most MMPs, induction by UVB peaked at 24 hours, although some expression is seen at 6 hours with some MMPs. MMP12 gene and mRNA expression by UVA1 is seen at 6 hours but primarily at 24 hours. The most striking fold increases with UVB were for MMP1 and MMP3 with array and qPCR technology. There was also an impressive increase of MMP10 mRNA with qPCR. Neither UVB nor UVA1 had any effect on MMP2 assessed by microarray, mRNA (data not shown), and protein. The most striking effect of UVA1 was on MMP12, as assessed by the three techniques. In contrast, UVB had little effect on MMP12 expression. There was modest or no evidence of any UVA1 induction of MMP3 or MMP10 by qPCR. MMP12 mRNA expression has been reported in human skin *in vivo* at 16 and 24 hours by others (Chung *et al.*, 2002), after a 2 minimal erythema dose (MED) exposure from a broad-spectrum UVB–UVA source. In another study, no MMP12 mRNA was

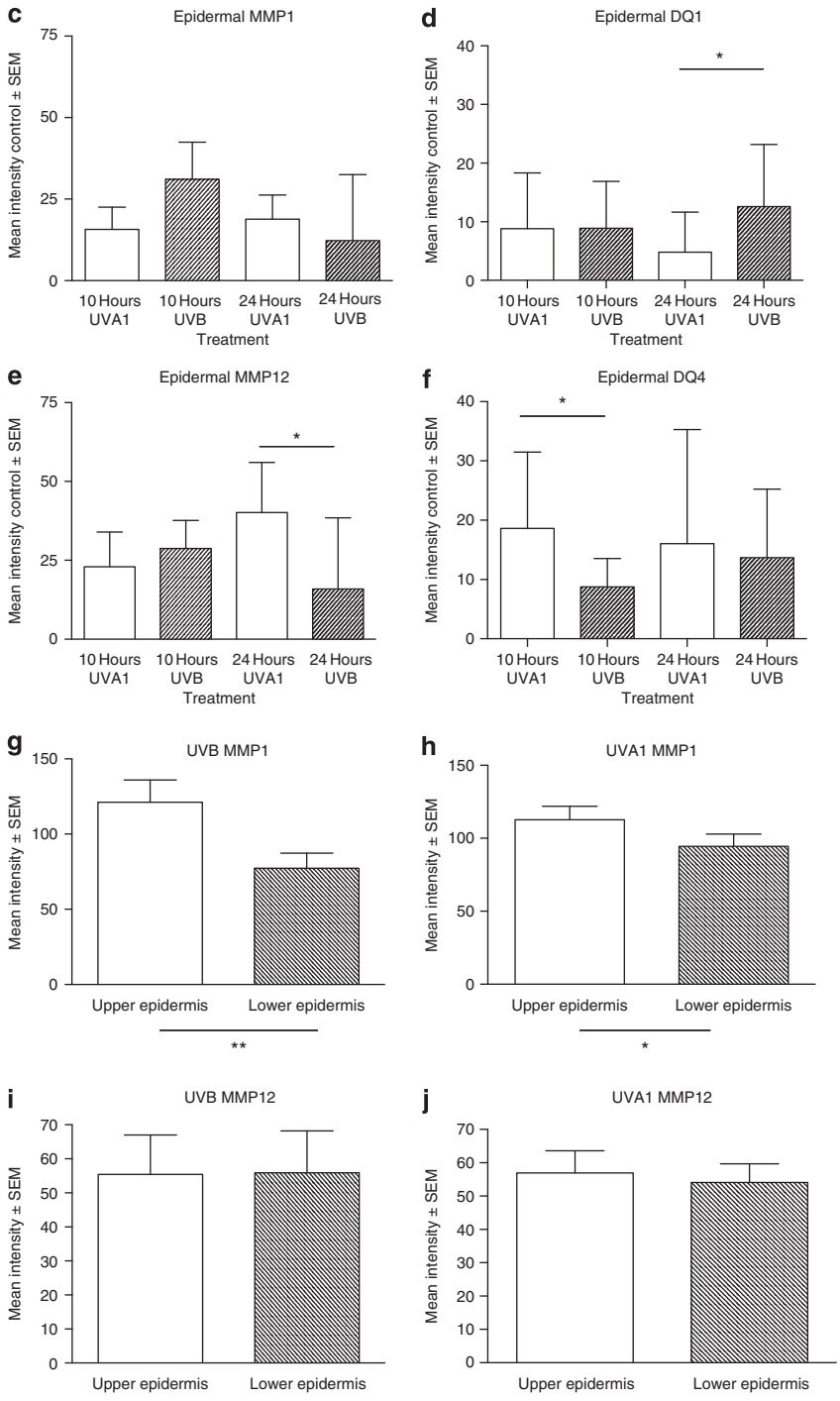

Figure 2. Continued.

detected after exposure to UVA1 or broad-spectrum UVR (UVB with UVA) for three consecutive days (Saarialho-Kere *et al.*, 1999).

Recently, it was shown that 40 J cm^{-2} UVA1 in human skin *in vivo* increased MMP1 and MMP3 mRNA by ~80- and 50-fold, respectively, at 24 hours (Wang *et al.*, 2013). These data are comparable to the responses we see to 50 J cm^{-2} UVA1 (especially for MMP3), which are 25- and 45-fold compared to nonirradiated control tissue for MMP1 and MMP3, respectively (see Figure 1c and d), given the error range in both data sets. Repeated (4 ×) low-dose UVA1 on human skin *in vivo* results in an accumulation of MMP1 and MMP3 mRNA expression (Wang *et al,* 2013).

Our protein studies show that UVR-induced MMPs are predominantly expressed in the epidermis, which is expected because of their role in epithelial tissue homeostasis after UVR

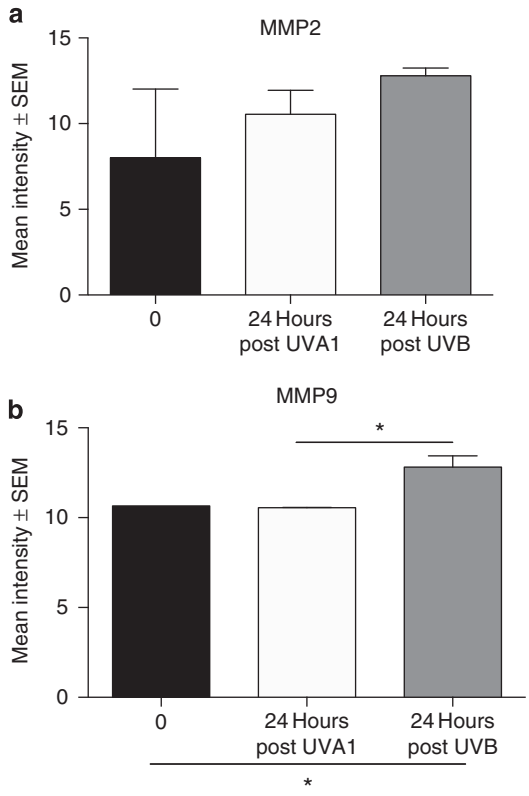

Figure 3. Effects of UVA1 and UVB on matrix metalloproteinase (MMP)2 and MMP9 expression in the epidermis. Using immunofluorescence there was (**a**) no differential MMP2 expression at 24 hours following 1 minimal erythema dose of UVA1 and UVB; (**b**) UVB induces significantly more MMP9 than UVA1 at 24 h. *$P \leqslant 0.05$; $n = 3$.

injury. Other studies support this observation (MMP1, MMP3, and MMP9 mRNA and MMP1 activity) after exposure to solar-simulating radiation (Quan *et al*, 2009). However, we believe that our data demonstrate MMP1 protein/activity by spectrally pure UVB and UVA1.

There are eight elastases in human skin (Liang *et al.*, 2006), of which human macrophage elastase (HME) or MMP12 (Shapiro, 1998; Shapiro *et al.*, 1993), and neutrophil-derived (neutrophil elastase (NE)) are induced by UVR (Lee *et al.*, 2008). NE has long-term photobiological significance, because NE-deficient mice are resistant to photoaging (Starcher and Conrad, 1995; Takeuchi *et al.*, 2010) and squamous cell carcinoma formation (Starcher *et al.*, 1996) after exposure to a source containing 10% UVB and 90% UVA. This suggests that elastase may be linked to these two long-term consequences of UVR exposure.

MMP12 protein has been detected in ''a few stromal fibroblast/macrophage-like cells'' after exposure to UVA1 (Saarialho-Kere *et al.*, 1999). A modest induction of dermal fibroblast MMP12 (but not in macrophages) has also been reported in human skin *in vivo* after a 2 MED exposure from a broad-spectrum UVB–UVA source (Chung *et al.*, 2002; Saarialho-Kere *et al.*, 1999). UVB induced significantly greater ($P = 0.02$) depletion of papillary dermal macrophages than did UVA1 (Figure 5b). Thus MMP12 could be explained by the larger presence of activated macrophages in the dermis after UVA1. MMP12-negative macrophages cannot penetrate the dermal/epidermal junction (Shipley *et al.*, 1996), and we found no evidence of epidermal macrophages. We did not stain for NE, but dermal neutrophil infiltration (and likely NE release) is a predominantly UVB-driven process (Lee *et al.*, 2008) and is therefore unlikely to explain our UVA1 data. MMP12 is also produced by activated T cells (Hughes *et al.*, 1998), transformed keratinocytes, and keratinocyte-derived tumors (Kerkela *et al.*, 2000). Given that we found MMP12 primarily in the epidermis, irrespective of spectrum, we suggest that it is mainly derived from normal keratinocytes. However, we cannot exclude the possibility that some MMP12 may be derived from dermal cells such as fibroblasts and macrophages.

UVB and UVA1 induced the degradation of DQ collagen type I. The assessment of specific MMP function by *in situ* zymography is complicated by cross-reactivity. DQ collagen type I is primarily a substrate for MMP1 but is also degraded by MMP2, MMP3, MMP9, and MMP13 (Yan and Blomme, 2003). There is no UVR induction of MMP2 mRNA or protein (Figure 3a) at 24 hours but there is a small (~20%) significant increase in MMP9 protein by UVB (Figure 3b). This has been previously reported (as 92 kd gelatinase) (Fisher *et al.*, 1997) with a broad-spectrum UVB–UVA source. As UVB and UVA1 induce MMP13 mRNA to similar extents (Figure 1h), it is possible that the UVB-induced collagen I hydrolytic activity is also mediated via MMP3 and MMP9. We did not measure MMP3 protein, but UVB resulted in a very large increase in its mRNA that was not seen with UVA1. However, MMP3 protein (stromelysin-1) has been shown to increase after exposure to a UVB–UVA source (Fisher *et al.*, 1997).

UVA1 was more effective than UVB for the degradation of DQ collagen type IV in the epidermis (10 hours). This is primarily a substrate for MMP12, but it is also hydrolyzed by MMP2, MMP7, MMP9, and MMP25 (Yan and Blomme, 2003). As MMP2, MMP7, MMP9, and MMP25 mRNAs were not induced by UVA1, the induction of enzyme activity is more likely to reflect the induction of MMP12. This is also supported by the lack of UVA1 effects on MMP2 and MMP9 protein expression. There is some collagen IV hydrolysis with UVB at 10 and 24 hours that could be due to UVB-induced MMP9 (Figure 3b), although without MMP12-blocking enzymes we cannot rule out the effects of MMP12.

UVB was significantly more effective than UVA1 at inducing epidermal MMP1 activity at 24 hours. In contrast, UVA1 was more potent at induction of epidermal MMP12 protein (24 hours) and its activity (10 hours) against DQ collagen type IV. It was also significantly better at the induction of dermal MMP1 and MMP12 proteins at 24 hours. This difference is likely to be due to greater UVA1 dermal penetration/scattering (Tewari *et al.*, 2011; Tewari *et al.*, 2012). Interestingly, we found no spectral differences for dermal MMP1 and MMP12 activity against their respective collagen substrates, but there was large interpersonal variation. There was some UVB-induced MMP12 protein, predominantly in the epidermis, but to a much lesser extent than with UVA1.

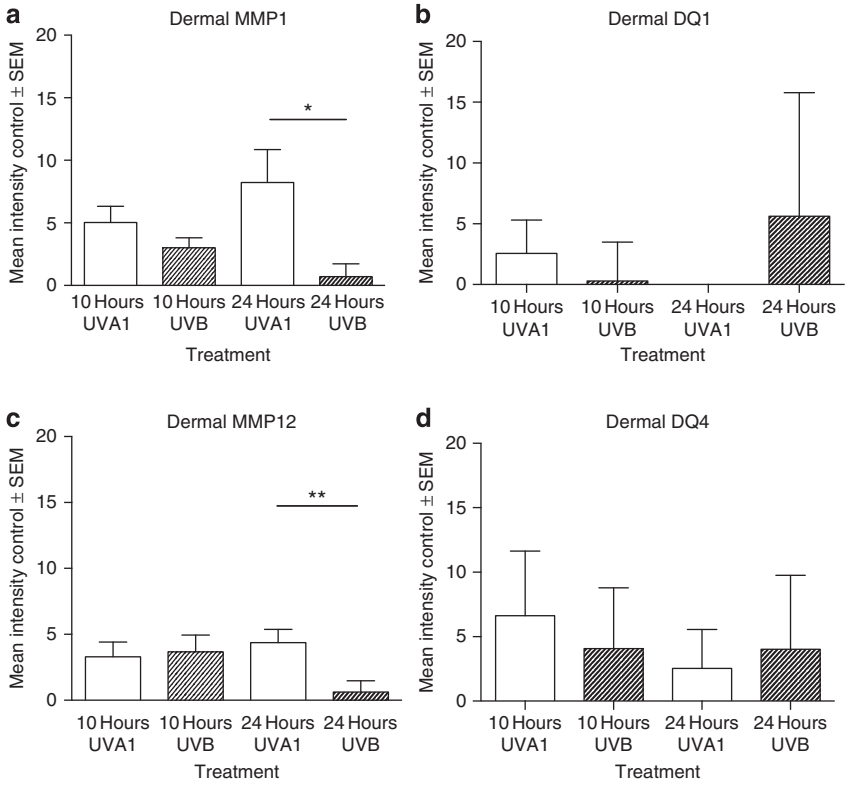

Figure 4. UVA1 induces more dermal metalloproteinase (MMP) protein than UVB. Using immunofluorescence and *in situ* zymography (**a**) there was more UVA1-induced MMP1 than UVB-induced MMP1 at 24 hours, (**b**) there were no spectral differences for the degradation of dermal DQ1, (**c**) there was more UVA1-induced MMP12 than UVB-induced MMP12 at 24 hours, and (**d**) there were no spectral differences for the degradation of dermal DQ4. Scale bar = 50 μm. $*P \leqslant 0.05$, $**P \leqslant 0.01$; $n = 3$.

The small amount (relative to epidermis) of UVA1-induced MMP12 in the dermis, maybe insufficient to degrade elastin at 24 hours because no elastase activity was detected in the dermal region, although this was considerable in the epidermal region. This may be attributed to greater MMP12 protein induction in the epidermis. This is also supported by the *in situ* zymography data for degradation of collagen IV. Essentially, the degradation of two substrates by MMP12 was an epidermal phenomenon in which UVA1 was more effective than UVB.

Dermal extracellular collagens are degraded in photoaging. Quan *et al* (2009) have suggested that epidermal MMP1, MMP3, and MMP9 (where they report the majority is synthesized), diffuse into the dermis to degrade collagen (Quan *et al.*, 2009). However, studies on photoaged skin show more MMPs in the dermis than in the epidermis (Chung *et al.*, 2002; Quan *et al.*, 2013). Overall, this suggests that repeated solar UVR exposure results in an accumulation of dermal MMP, whether by diffusion from the epidermis or by a gradual accumulation of dermally synthesized protein. It is also possible that our 24-hour sampling time was not optimal for MMP12 diffusion from the epidermis into the dermis and the degradation of elastin.

Light microscopy shows that the papillary dermis of photoaged skin contains an accumulation of amorphous disorganized elastin fibrils, which is known as ''solar elastosis'' (Calderone and Fenske, 1995; Yaar and Gilchrest, 2007). In its early stages, there is an accumulation of insoluble disorganized elastin and microfibrillar proteins (fibronectin) (Chen *et al.*, 1986; Lavker and Kligman, 1988), seen clinically as waxy, thickened, and furrowed facial skin. In more advanced solar elastosis, degeneration of dermal elastin results in a mottled appearance that is clinically associated with a loss of skin elasticity. The addition of an elastase to an elastin culture (Braverman and Fonferko, 1982) results in a mottled appearance that is similar to that seen in late solar elastosis. This appearance, termed ''zebra bodies'', can also be induced by repeated erythemal doses of UVA, but not by solar-simulated radiation (Kumakiri *et al.*, 1977).

Our data with gene array, qPCR, and protein activity strongly suggest that UVA1 preferentially induces MMP12. We acknowledge that our protein studies are based on small sample sizes ($n = 3$). However, they generally correlate with the mRNA data, and the protein expression and activity data are mutually consistent. Our elastase activity data (Figure 5) are particularly striking. MMP12 protein has been observed in the upper dermis of patients with solar elastosis and actinic keratosis (Saarialho-Kere *et al.*, 1999; Chung *et al.*, 2002). Chung *et al.* (2002) suggest that MMP12 has a role in elastin remodeling in solar elastosis of the face. We suggest that solar

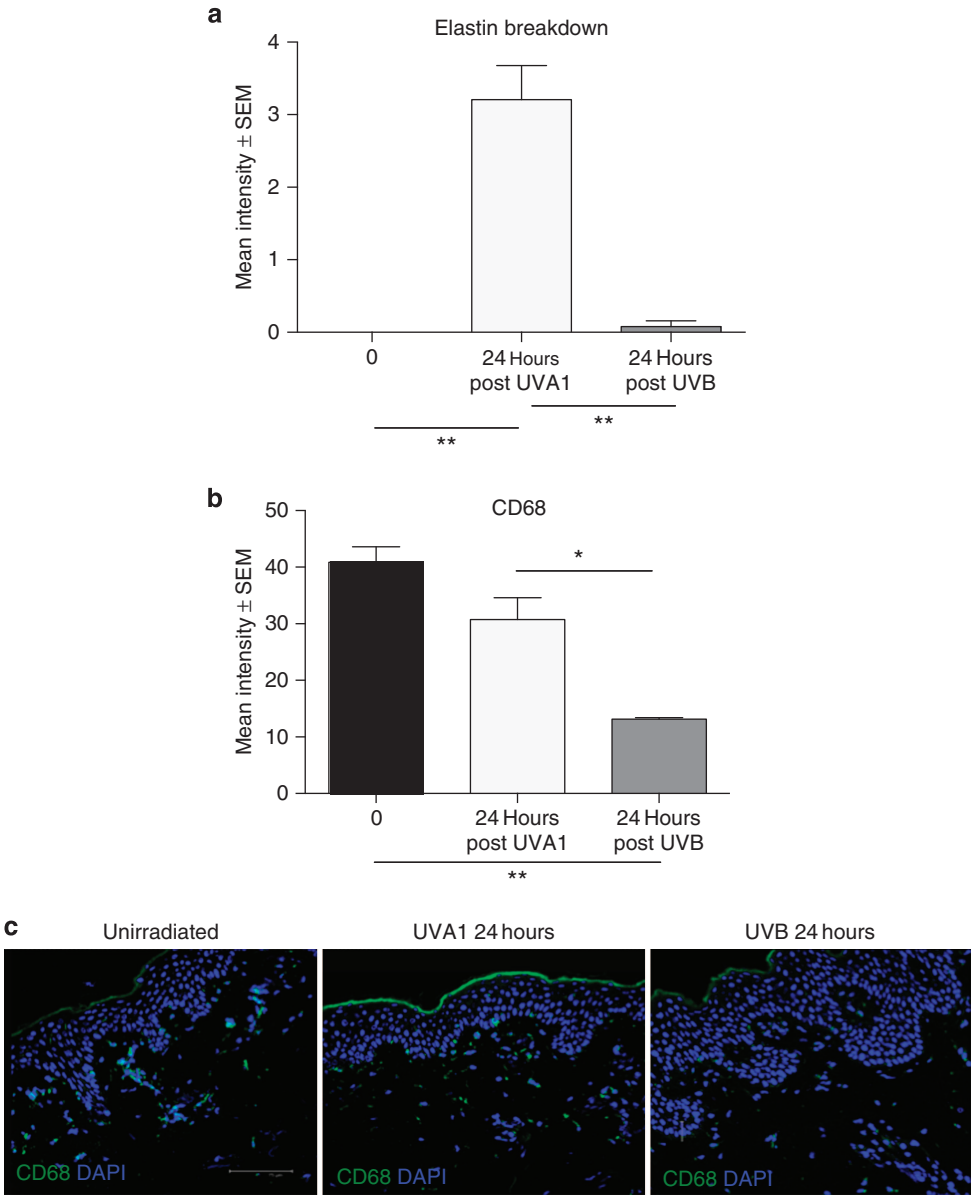

Figure 5. (a) UVA1, but not UVB, initiates DQ elastin degradation. *In situ* zymography showing fluorescence proportional to elastin breakdown at 24 hours after a 1 minimal erythema dose exposure. (**b**) No UVA1 induced depletion of macrophages. Immunofluorescence shows higher numbers of $CD68^{+}$ macrophages present in the dermis at 24 hours after UVA1 than UVB, (**c**) examples of CD68-positive staining in one individual. $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$; $n = 3$. Scale bar = 50 μm.

elastosis, and its association with MMP12, may be explained by our studies because MMP12 is the major enzyme for the degradation of elastin (Gronski *et al.*, 1997; Woessner, 1991). However, we cannot exclude other MMPs having a role in the pathogenesis of solar elastosis, because MMP2, MMP7, and MMP9 also have elastolytic activity and may be induced by UVB (e.g., MMP9 as shown in Figure 3b). In addition, MMP12 degrades other substrates apart from type IV collagen including laminin 1, fibronectin, vitronectin, and proteoglycans (Gronski *et al.*, 1997).

We hypothesize that solar UVA1 induces the expression of MMP12, which then degrades elastin, contributing to the loss of skin elasticity seen in late solar elastosis. This may occur, as suggested by Quan *et al* (2009), by diffusion of the MMPs from the epidermis to the upper dermis. The resultant loss of elasticity, termed ''sagging'', has been shown to be a UVA1-dependent process in hairless mice, with an action spectrum peak at 340 nm (Bissett *et al.*, 1987, 1989). This suggests that UVA1 has important implications for photoaging and also possibly photocarcinogenesis (Starcher and Conrad, 1995; Starcher *et al.*, 1996). This is supported by studies that show that people habitually exposed to UVA through glass on one side of the face show more signs of photoaging and skin cancer on the exposed side (Butler and Fosko, 2010; Foley *et al.*, 1986; Mac-Mary *et al.*, 2010; Singer *et al.*, 1994).

Table 1. Volunteer demographics and their just-perceptible MED

Study	Skin type	Sex	Age	MED UVA1 (J cm^{-2})	MED UVB (mJ cm^{-2})
UVA1 time course	I	M	27	48.8	30.0
	II	M	21	48.8	30.0
	I	F	21	61.8	37.0
	I	F	21	61.8	23.0
Mean ± SD		M + F	**22.5 ± 2.6**	**55.3 ± 6.5**	**30.0 ± 4.9**
UVA1 and UVB comparision	I	M	20	61.1	19.0
	I	M	21	61.1	23.0
	II	F	23	61.1	30.0
	I	F	22	61.1	23.0
	II	F	28	76.2	30.0
Mean ± SD		M + F	**22.8 ± 3.1**	**64.1 ± 6.8**	**25.0 ± 4.8**
Protein validation	II	F	24	61.1	37.0
	II	F	27	61.1	37.0
	I	F	22	48.8	30
Mean ± SD			**24.3 ± 2.5**	**57.0 ± 7.1**	**34.7 ± 4.0**
Combined mean ± SD	7I, 5II	4M, 8F	**23.0 ± 2.8**	**59.4 ± 7.7**	**29.1 ± 6.1**

Abbreviations: F, female; M, male; MED, minimal erythema dose.

A reduction of photoaging in an intense solar environment has been observed with the long-term discretionary use of a sunscreen (SPF 15 +)(Hughes *et al.*, 2013). Its formulation is not photostable, and would have lost most of its UVA1 protection within 1 hour (Dr B Herzog, personal communication). Our MMP12 data support the inclusion of good photostable UVA1 protection in sunscreens to enhance their ability to inhibit photoaging. Furthermore, our data suggest that the inhibition of MMP12 may be a future strategy for protecting against photoaging.

The absorption of UVR by chromophores mediates all photobiological reactions. UVB absorbed by DNA results in CPD formation, the action spectrum of which peaks at 300 nm in human skin *in vivo* (Young *et al.*, 1998). There is evidence that DNA is a chromophore for MMP1 via CPD formation (Dong *et al.*, 2008). Our recent findings (Tewari *et al.*, 2012; Tewari *et al.*, 2011) showed considerable attenuation of UVB-induced CPDs with skin (epidermis and dermis) depth, which was not the case with UVA1. Thus, we would expect to see marked attenuation of UVB-induced MMP1 with skin depth, as shown in Figures 2g and 4a, if DNA were the putative chromophore. In contrast, epidermal depth has no effect on the distribution of MMP12 induced by UVB or UVA1 (Figure 2i and j). This suggests that they have different chromophores. We therefore propose that UVA1 induces MMP12 via a non-DNA chromophore that generates reactive oxygen species (Scharffetter-Kochanek *et al.*, 1993; Wlaschek *et al.*, 1995). This is supported by studies that showed that topical reactive oxygen species scavengers reduced MMP12 mRNA in human skin (Chung *et al.*, 2002). UVA1 did not induce MMP3 or MMP10 when assessed by qPCR. Our erythemal dose of UVA1 (50 J cm^{-2}) does induce CPDs, although at one-fourth to one-third times lower levels than after an erythemally equivalent exposure to UVB (Tewari, Sarkany *et al.*, 2011). This suggests that these enzymes may not only have a CPD threshold but may also have non-DNA chromophores that are required for their induction.

MATERIALS AND METHODS

Volunteers

The studies were approved by the St Thomas' Hospital, London, UK Ethics Committee (Ref: 09/H0802/98) in accordance with the declaration of Helsinki. The details of the 12 healthy skin type I/II volunteers are shown in Table 1. Participants gave written informed consent before taking part in the study.

Irradiation

UVR sources, dosimetry, and irradiation protocol. Emission spectra and irradiances of the UVA1 and UVB sources, and assessment of MED are previously described (Tewari *et al.*, 2011). Table 1 shows that the mean MEDs for 12 volunteers were 29.2 ± 5.8 mJ cm^{-2} (UVB) and 58.6 ± 7.9 J cm^{-2} (UVA1).

Experimental protocol. (i) ''UVA1 time course'': four skin type I/II volunteers were irradiated over a 1-cm^2 area on previously unexposed buttock skin with 50 J cm^{-2} UVA1 and 4-mm punch biopsies were taken under local anesthesia 6 and 24 hours later. This was ~1MED (minimal erythema dose) and was based on our previous work (Tewari *et al.*, 2011). (ii) ''UVA1 and UVB comparison'': five skin type I/II participants were exposed to 50 J cm^{-2} UVA1 and 30 mJ cm^{-2} (~1MED) UVB and biopsies were taken at 6 and at 24 hours. (iii) ''Protein validation'':

a further three skin type I/II individuals were recruited, had UVR sensitivity tested as above, and received UVB and UVA1 irradiations equivalent to ~1MED (30 mJ cm^{-2} UVB and 50 J cm^{-2} UVA1). Biopsies were taken at 10 and 24 hours along with an nonirradiated control biopsy.

Microarray. A single color hybridization on Agilent Whole Human Genome Oligo Microarrays (Agilent Technologies, Waldbronn, Germany) (4 × 44 K) and bioinformatics was performed (Milteyni Biotech, Bergisch Gladbach, Germany). Briefly, biopsies were snap-frozen in liquid nitrogen and RNA was extracted (Trizol, Sigma, St Louis, MO) and quality checked (Agilent 2100 bioanalyzer platform; Agilent Technologies). cRNA was produced, Cy3 labeled, and hybridized overnight (~17 hours, 65 °C) to Agilent Whole Genome Oligo Microarray chips (4 × 44 K) using the Agilent Gene Expression Hybridzation Kit (Agilent Technologies). Fluorescence signals were detected using Agilent's Microarray Scanner system, Agilent Feature Extraction Software was used to process intensities. Raw intensity data were extracted from Feature Extraction output files using Rosetta Resolver software (Rosetta, Inpharmatics, Kirkland, WA). Background-corrected intensity values were normalized between arrays using quantile normalization (Bolstad *et al.*, 2003). Log_2-transformed normalized intensity values were used for subsequent statistical analysis. Quality controls include comparison of intensity profiles and a global correlation analysis. The microarray data is deposited at NCBI GEO with accession number GSE45493.

Quantitative real-time PCR

Total RNA was converted to cDNA (Applied Biosystems, Paisley, UK) and quality controlled using reverse transcription PCR with β-actin as the housekeeping gene. cDNA was visualized as characteristic bands on a 3% agarose gel under UVR. qPCR was performed using Taqman Gene Expression Assays (Applied Biosystems) according to the manufacturer's protocol with the following TaqMan DNA probes (Applied Biosystems), FAM (6-carboxyfluorescein) labeled, and GAPDH (Applied Biosystems) housekeeping gene, VIC labeled. Probes used (gene name and reference no): MMP1 Hs00899658_m1, MMP3 Hs00968305_m1, MMP9 Hs00234579_m1, MMP10 Hs00233987_m1, MMP12 Hs00899668_m1. Fold change was calculated using the ΔΔCT method.

Protein validation

Biopsies were placed in OCT (VWR Chemicals, Leuven, Belgium), embedded in isopentane (VWR Chemicals, Fontenay-sous-Bois, France), and once placed in cryovials were then frozen in liquid nitrogen. 5–7 μm sections were mounted on Superfrost plus slides and stored at −80 °C. Sections were placed at room temperature (10 minutes), fixed in cold acetone (10 minutes), then rinsed in phosphate-buffered saline (PBS) for 5 minutes. (i) Immunofluorescence: sections were incubated with blocking buffer for 20 minutes (10% goat serum (DAKO, Cambridge, UK) 0.1% BSA, 0.1% Tween-20 in PBS), washed in PBS, incubated for 90 minutes with MMP1 (1:100), MMP12 (1:400) (recognizes the proactive and active forms of MMP12), rabbit antihuman antibodies (Abcam, UK), CD68 (1:100) mouse antihuman antibody (Abcam, Cambridge, UK) washed in PBS, and incubated with either Alexa Fluor goat antirabbit 555 or goat antimouse 555 (Invitrogen, Paisley, UK) at 1:200 for 30 minutes, counterstained with prolong gold antifade with DAPI (Molecular Probes, Paisley, UK), coverslipped, and stored away from light. Imaging was performed with a Zeiss Axiophot microscope (Harpenden, UK) and Nikon DS-U2 camera (Kingston upon Thames, UK). Images were captured in 2560 × 1920 format, gain × 1.00, 4 second exposure. (ii) *in situ* zymography: slides were incubated with 60 μl substrate (low-gelling agarose (Sigma, Dorset, UK) (1 g dissolved in 500 ml PBS)), with four drops of 4′,6-diamidino-2-phenylindole with antigold fade (Invitrogen) and DQ-collagen 1, DQ-collagen 4, or DQ elastin (all from Molecular Probes) at 1:10 for ~18 hours in the dark. Fluorescent imaging for fluorescein (green) and 4′,6-diamidino-2-phenylindole (blue) was performed. Images were obtained as above and captured in 2560 × 1920 format, gain × 1.00, 1 second exposure. Analysis was performed using NIS elements BRv2 software package as previously described (Tewari *et al.*, 2011) after removing background control intensity. For the spread of MMP1 and MMP12 across the epidermis, thresholding was set to capture red intensity, and upper epidermis and lower epidermis were visually gated to give mean intensity values corresponding to MMP amount.

Data analysis

For microarray data, bioinformatical analysis was based on normalized Log_2 intensities using R/Bioconductor and software packages therein (http://www.R-project.org; http://www.bioconductor.org). Analysis of variance with repeated measurements followed by Tukey *post hoc* tests was used to test for expression differences among the groups. The UVA1 time course was as follows: UVA1 6 hours versus UVA1 24 hours (50 J cm^{-2} doses), UVA1 versus UVB at 6 hours, and UVA1 versus UVB at 24 hours. All *P*-values were adjusted for multiple testing (Benjamini and Hochberg, 1995). Differentially expressed genes were considered if both the analysis of variance and Tukey *post hoc* test *P*-values were $\leqslant 0.05$ (adjusted *P*-value or false discovery rate $\leqslant 0.3$) and the expression difference was at least twofold.

Functional analysis of candidate genes was performed using DAVID (Database for Annotation, Visualization and Integrated Discovery) v6.7 (Dennis *et al.*, 2003) to identify biological clusters and GeneGo Metacore v7 to identify key pathways. Briefly, gene ratio lists for pooled individuals (fold change $\geqslant 2$, $P \leqslant 0.05$) from the 6- and 24-hour biopsies were uploaded to the software program, which generated pathway maps and *P*-values associating the statistical likelihood of a sequence of genes with a particular pathway. All graphs were generated using the Graphpad Prism v4 statistics package.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. Funding was also received from La Roche-Posay Pharmaceuticals, and Angela Tewari was awarded a Medical Research Council (MRC)/British Skin Foundation (BSF) clinical training fellowship during which the above research was completed. We are grateful for assistance from Graham Harrison and also Rachel Watson and Abigail Langton with *in situ* zymography techniques. We thank Bernd Herzog of BASF for his input on the photodegradation of the sunscreen mentioned in the discussion. The microarray data are deposited at NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE45493.

REFERENCES

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289–300

Bernhard G, Mayer B, Seckmeyer G (1997) Measurements of spectral solar UV irradiance in tropical Australia. *J Geophys Res* 102:8719–30

Bissett DL, Hannon DP, Orr TV (1987) An animal model of solar-aged skin: histological, physical, and visible changes in UV-irradiated hairless mouse skin. *Photochem Photobiol* 46:367–78

Bissett DL, Hannon DP, Orr TV (1989) Wavelength dependence of histological, physical, and visible changes in chronically UV-irradiated hairless mouse skin. *Photochem Photobiol* 50:763–9

Bolstad BM, Irizarry RA, Astrand M *et al.* (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19:185–93

Braverman I, Fonferko E (1982) Studies in cutaneous aging: 1. The elastic fiber network. *J Invest Dermatol* 78:434–43

Brenneisen P, Oh J, Wlaschek M *et al.* (1996) Ultraviolet B wavelength dependence for the regulation of two major matrix-metalloproteinases and their inhibitor TIMP-1 in human dermal fibroblasts. *Photochem Photobiol* 64:877–85

Bruls WA, Slaper H, van der Leun JC *et al.* (1984) Transmission of human epidermis and stratum corneum as a function of thickness in the ultraviolet and visible wavelengths. *Photochem Photobiol* 40:485–94

Butler ST, Fosko SW (2010) Increased prevalence of left-sided skin cancers. *J Am Acad Dermatol* 63:1006–10

Calderone DC, Fenske NA (1995) The clinical spectrum of actinic elastosis. *J Am Acad Dermatol* 32:1016–24

Chakraborti S, Mandal M, Das S *et al.* (2003) Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* 253:269–85

Chen VL, Fleischmajer R, Schwartz E *et al.* (1986) Immunochemistry of elastotic material in sun-damaged skin. *J Invest Dermatol* 87:334–7

Choi W, Miyamura Y, Wolber R *et al.* (2010) Regulation of human skin pigmentation in situ by repetitive UV exposure: molecular characterization of responses to UVA and/or UVB. *J Invest Dermatol* 130:1685–96

Chung JH, Seo JY, Lee MK *et al.* (2002) Ultraviolet modulation of human macrophage metalloelastase in human skin *in vivo*. *J Invest Dermatol* 119:507–12

Dennis G Jr., Sherman BT, Hosack DA *et al.* (2003) DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol* 4:P3

Dong KK, Damaghi N, Picart SD *et al.* (2008) UV-induced DNA damage initiates release of MMP-1 in human skin. *Exp Dermatol* 17:1037–44

Enk CD, Jacob-Hirsch J, Gal H *et al.* (2006) The UVB-induced gene expression profile of human epidermis *in vivo* is different from that of cultured keratinocytes. *Oncogene* 25:2601–14

Enk CD, Shahar I, Amariglio N *et al.* (2004) Gene expression profiling of *in vivo* UVB-irradiated human epidermis. *Photodermatol Photoimmunol Photomed* 20:129–37

Fisher GJ, Datta SC, Talwar HS *et al.* (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* 379:335–9

Fisher GJ, Quan T, Purohit T *et al.* (2009) Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *Am J Pathol* 174:101–14

Fisher GJ, Wang ZQ, Datta SC *et al.* (1997) Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337:1419–28

Foley P, Lanzer D, Marks R (1986) Are solar keratoses more common on the driver's side? *Br Med J* 293:18

Gronski TJ Jr., Martin RL, Kobayashi DK *et al.* (1997) Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase. *J Biol Chem* 272:12189–94

Herrmann G, Wlaschek M, Lange TS *et al.* (1993) UVA irradiation stimulates the synthesis of various matrix-metalloproteinases (MMPs) in cultured human fibroblasts. *Exp Dermatol* 2:92–7

Hughes MC, Williams GM, Baker P *et al.* (2013) Sunscreen and prevention of skin aging: a randomized trial. *Ann Intern Med* 158:781–90

Hughes PM, Wells GM, Clements JM *et al.* (1998) Matrix metalloproteinase expression during experimental autoimmune neuritis. *Brain* 121:481–94

Kerkela E, Ala-Aho R, Jeskanen L *et al.* (2000) Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. *J Invest Dermatol* 114:1113–9

Kerkela E, Saarialho-Kere U (2003) Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. *Exp Dermatol* 12:109–25

Kerr AC, Ferguson J, Attili SK *et al.* (2012) Ultraviolet A1 phototherapy: a British Photodermatology Group workshop report. *Clin Exp Dermatol* 37:219–26

Kumakiri M, Hashimoto K, Willis I (1977) Biologic changes due to long-wave ultraviolet irradiation on human skin: ultrastructural study. *J Invest Dermatol* 69:392–400

Lavker RM, Kligman AM (1988) Chronic heliodermatitis: a morphologic evaluation of chronic actinic dermal damage with emphasis on the role of mast cells. *J Invest Dermatol* 90:325–30

Lee PL, van Weelden H, Bruijnzeel PL (2008) Neutrophil infiltration in normal human skin after exposure to different ultraviolet radiation sources. *Photochem Photobiol* 84:1528–34

Liang J, Liu E, Yu Y *et al.* (2006) Macrophage metalloelastase accelerates the progression of atherosclerosis in transgenic rabbits. *Circulation* 113: 1993–2001

Mac-Mary S, Sainthillier JM, Jeudy A *et al.* (2010) Assessment of cumulative exposure to UVA through the study of asymmetrical facial skin aging. *Clin Interv Aging* 5:277–84

Mouret S, Baudouin C, Charveron M *et al.* (2006) Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc Natl Acad Sci USA* 103:13765–70

Quan T, Little E, Quan H *et al.* (2013) Elevated matrix metalloproteinases and collagen fragmentation in photodamaged human skin: impact of altered extracellular matrix microenvironment on dermal fibroblast function. *J Invest Dermatol* 133:1362–6

Quan T, Qin Z, Xia W *et al.* (2009) Matrix-degrading metalloproteinases in photoaging. *J Investig Dermatol Symp Proc* 14:20–4

Saarialho-Kere U, Kerkela E, Jeskanen L *et al.* (1999) Accumulation of matrilysin (MMP-7) and macrophage metalloelastase (MMP-12) in actinic damage. *J Invest Dermatol* 113:664–72

Sage E, Lamolet B, Brulay E *et al.* (1996) Mutagenic specificity of solar UV light in nucleotide excision repair-deficient rodent cells. *Proc Natl Acad Sci USA* 93:176–80

Scharffetter K, Wlaschek M, Hogg A *et al.* (1991) UVA irradiation induces collagenase in human dermal fibroblasts *in vitro* and *in vivo*. *Arch Dermatol Res* 283:506–11

Scharffetter-Kochanek K, Wlaschek M, Briviba K *et al.* (1993) Singlet oxygen induces collagenase expression in human skin fibroblasts. *FEBS Lett* 331:304–6

Shapiro SD (1998) Matrix metalloproteinase degradation of extracellular matrix: biological consequences. *Curr Opin Cell Biol* 10:602–8

Shapiro SD, Kobayashi DK, Ley TJ (1993) Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. *J Biol Chem* 268:23824–9

Shipley JM, Wesselschmidt RL, Kobayashi DK *et al.* (1996) Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc Natl Acad Sci USA* 93:3942–6

Singer RS, Hamilton TA, Voorhees JJ *et al.* (1994) Association of asymmetrical facial photodamage with automobile driving. *Arch Dermatol* 130:121–3

Starcher B, Conrad M (1995) A role for neutrophil elastase in the progression of solar elastosis. *Connect Tissue Res* 31:133–40

Starcher B, O'Neal P, Granstein RD *et al.* (1996) Inhibition of neutrophil elastase suppresses the development of skin tumors in hairless mice. *J Invest Dermatol* 107:159–63

Steenport M, Khan KM, Du B *et al.* (2009) Matrix metalloproteinase (MMP)-1 and MMP-3 induce macrophage MMP-9: evidence for the role of TNF-alpha and cyclooxygenase-2. *J Immunol* 183:8119–27

Takeuchi H, Gomi T, Shishido M *et al.* (2010) Neutrophil elastase contributes to extracellular matrix damage induced by chronic low-dose UV irradiation in a hairless mouse photoaging model. *J Dermatol Sci* 60: 151–8

Tewari A, Lahmann C, Sarkany R *et al.* (2012) Human erythema and matrix metalloproteinase-1 mRNA induction, *in vivo*, share an action spectrum which suggests common chromophores. *Photochem Photobiol Sci* 11:216–23

Tewari A, Sarkany RP, Young AR (2012) UVA1 induces cyclobutane pyrimidine dimers but Not 6-4 photoproducts in human skin *in vivo*. *J Invest Dermatol* 132:394–400

Uitto J (2008) The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. *J Drugs Dermatol* 7:s12–6

Walker SL, Young AR (2007) An action spectrum (290–320 nm) for TNFalpha protein in human skin *in vivo* suggests that basal-layer epidermal DNA is the chromophore. *Proc Natl Acad Sci USA* 104:19051–4

Wang F, Smith NR, Tran BA *et al.* (2013) Dermal damage promoted by repeated low-level UV-A1 exposure despite tanning response in human skin. *JAMA* 150:401–6

Wlaschek M, Briviba K, Stricklin GP *et al.* (1995) Singlet oxygen may mediate the ultraviolet A-induced synthesis of interstitial collagenase. *J Invest Dermatol* 104:194–8

Wlaschek M, Heinen G, Poswig A *et al.* (1994) UVA-induced autocrine stimulation of fibroblast-derived collagenase/MMP-1 by interrelated loops of interleukin-1 and interleukin-6. *Photochem Photobiol* 59:550–6

Woessner JF Jr. (1991) Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 5:2145–54

Woollons A, Kipp C, Young AR *et al.* (1999) The 0.8% ultraviolet B content of an ultraviolet A sunlamp induces 75% of cyclobutane pyrimidine dimers in human keratinocytes *in vitro*. *Br J Dermatol* 140:1023–30

Yaar M, Gilchrest BA (2007) Photoageing: mechanism, prevention and therapy. *Br J Dermatol* 157:874–87

Yan SJ, Blomme EA (2003) *In situ* zymography: a molecular pathology technique to localize endogenous protease activity in tissue sections. *Vet Pathol* 40:227–36

Young AR, Chadwick CA, Harrison GI *et al.* (1998) The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *Br J Dermatol* 111:982–8