Individual Photosensitivity of Human Skin and UVA-Induced Pyrimidine Dimers in DNA

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Delineation of the DNA-damaging properties of UVA radiation is a major issue in understanding solar carcinogenesis. Emphasis was placed in this study on the formation of cyclobutane pyrimidine dimers (CPDs), which are now well established as the most frequent UVA-induced DNA lesions in human skin. The yield of CPDs was determined by a chromatographic assay following *ex vivo* UVA and UVB irradiation of biopsies taken from either phototype II or IV volunteers. A clear correlation was found between the frequency of UVB-induced CPDs and both the phototype and the minimum erythemal dose (MED). Similar results were obtained for the induction of CPDs upon exposure to UVA. Moreover, an excellent correlation was observed for each donor between the yield of DNA damage induced by either UVB or UVA. These observations show that the key parameters driving UVA-induced formation of CPDs are attenuation of radiation in the skin and the number of photons reaching skin cells rather than the cellular content in photosensitizers. In addition, the results show that both MED and phototype are good predictors of the vulnerability of DNA toward UVB and UVA in the skin. This result is of importance for the identification of individuals to be extensively protected.

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INTRODUCTION

Induction of damage to DNA is a key initiating event in the induction of skin cancer by solar radiation, and in particular the most deleterious portion of its spectrum in the UV range (Melnikova and Ananthaswamy, 2005). Emphasis has been placed on UVB radiation that overlaps with DNA absorption and triggers the formation of the well-known pyrimidine dimers. UVA represents the less energetic portion of solar UV and is less biologically active than UVB. Yet, involvement of UVA in skin carcinogenesis is a growing concern for several reasons. First, UVA was found to be mutagenic in cultured cells (Drobetsky *et al.*, 1995; Stary *et al.*, 1997; Rochette *et al.*, 2003; Kappes *et al.*, 2006) and tumorogenic in mice (de Laat *et al.*, 1997a, 1997b; Pastila and Leszczynski, 2005). UVA was also proposed by some authors to be involved in the induction of malignant melanoma (Setlow *et al.*, 1993; Setlow, 1999; Wang *et al.*, 2001), in agreement with the recent hypothesis that the increasing incidence of melanoma, particularly in young women, reflects their increased intermittent use of UVA-rich sunlamps (Coelho and Hearing, 2010). It should also be kept in mind that UVA is 20 to 100 times more abundant than UVB in solar light, depending on time of the day, latitude, and altitude. Moreover, although large improvements were made as the result of more severe regulations, sunscreens still provide a better protection in the UVB than in the UVA range. Exposure to natural UVA thus represents a rather large dose. To this must be added exposure resulting from the increasing use of artificial tanning equipment using UVA-rich sources. Altogether, the question is raised of the impact of UVA radiation on public health. The recent decision of the International Agency for Research on Cancer to classify artificial UV devices as carcinogens (El Ghissassi *et al.*, 2009) further emphasizes the carcinogenic risk associated with UVA.

It appears that a deeper understanding of the carcinogenic effects of UVA is needed. Physiological responses such as inflammation and immunodepletion (Krutmann, 1998; Halliday, 2005) can be put forward. Another likely explanation is the induction of damage to DNA. The genotoxic effects of UVA are most often described in terms of oxidative damage induced by photosensitization reactions (Wondrak *et al.*, 2006; Cadet *et al.*, 2009) and worsened by the release of iron from ferritin (Pourzand *et al.*, 1999). However, UVA-induced DNA damage cannot be limited to oxidative lesions and cyclobutane pyrimidine dimers (CPDs) also have to be considered. CPDs are the major dimeric lesions produced upon UVB irradiation, together with pyrimidine (6–4) photoproducts (64PPs; Cadet *et al.*, 2005). These photoproducts may be produced at any of the four bipyrimidine dinucleotides (TT, TC, CT, and CC), although with drastically different frequencies (Douki and Cadet, 2001). The strong

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Abbreviations: CPD, cyclobutane pyrimidine dimer; UVA-MED, minimum erythemal dose determined after exposure to UVA radiation; UVB-MED, minimum erythemal dose determined after exposure to simulated solar radiation; 64PP, pyrimidine (6–4) pyrimidone photoproduct

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mutagenic impact of CPDs is well documented in cultured cells (You *et al.*, 2001) and in human skin tumors (Brash *et al.*, 1991; Dumaz *et al.*, 1993; Ziegler *et al.*, 1993).

However, a number of evidence show that formation of pyrimidine dimers is not limited to the most energetic UV radiation and that they may also be produced by UVA as observed in bacteria (Tyrrell, 1973), cultured mammalian cells (Freeman and Ryan, 1990; Kielbassa *et al.*, 1997; Kvam and Tyrrell, 1997; Perdiz *et al.*, 2000; Douki *et al.*, 2003; Courdavault *et al.*, 2004), and skin (Freeman *et al.*, 1989; Young *et al.*, 1998b; Mouret *et al.*, 2006). The UVA photochemistry of DNA was found to be rather specific. In contrast to UVB, UVA does not induce the formation of 64PPs (Perdiz *et al.*, 2000; Douki *et al.*, 2003; Courdavault *et al.*, 2004). Among the CPDs formed, a predominance of TT cyclobutane dimer, much larger in proportion than upon UVB irradiation, is observed for UVA (Douki *et al.*, 2003; Rochette *et al.*, 2003; Courdavault *et al.*, 2004). A 10-fold lower yield of TC and CT CPD is observed, whereas the CC derivative is not detected (Douki *et al.*, 2003; Courdavault *et al.*, 2004). UVA-induced formation of CPDs may be explained by triplet energy transfer photosensitization reactions as documented for a series of molecules (Charlier and Hélène, 1967; Traynor and Gibbs, 1999; Lhiaubet *et al.*, 2001; Sauvaigo *et al.*, 2001; Lhiaubet-Vallet *et al.*, 2004). An alternative pathway is a direct photoreaction resulting from the low but significant absorption of UVA photons by DNA (Sutherland and Griffin, 1981; Mouret *et al.*, 2010). Observations that CPDs can be induced in isolated naked DNA upon UVA irradiation (Quaite *et al.*, 1992; Zhang *et al.*, 1997; Kuluncsics *et al.*, 1999; Jiang *et al.*, 2009) with the same efficiency and distribution than in cells (Kuluncsics *et al.*, 1999; Perdiz *et al.*, 2000) make the direct process more likely.

The relevance of CPDs to UVA genotoxicity is emphasized by several other observations. First, the yield of TT CPD was found to be larger than that of 8-oxo-7,8-dihydro-2′-deoxyguanosine, the main oxidative damage, in both cultured cells (Kielbassa *et al.*, 1997; Douki *et al.*, 2003; Courdavault *et al.*, 2004) and skin (Mouret *et al.*, 2006). CPDs are thus the main class of UVA-induced DNA lesions. In addition to these results related to the formation of the damage, observations made in recent mutagenesis studies of a majority of mutational events at bipyrimidine sites in UVA-irradiated human cells (Rochette *et al.*, 2003; Kappes *et al.*, 2006) strengthen the involvement of CPDs in UVA genotoxicity. An additional worsening aspect is the preliminary observation of a decrease in repair efficiency of CPDs following UVA irradiation (Courdavault *et al.*, 2005; Mouret *et al.*, 2006).

This series of recent observations supports a role of UVA-induced CPDs in solar carcinogenesis and raises the question of individual sensitivity to this genotoxic process. The modulation of the formation of CPDs by the skin type upon UVA irradiation may be different whether one formation mechanism is involved or the other. Efficiency of photosensitization depends on the amounts and cellular location of the sensitizing chromophore(s). These two last parameters are not necessarily less in favor of DNA damage induction in dark skin where the photosensitizer load may be higher than in fair skin. In such a case, phototype would not be correlated to the yield of CPDs in UVA-irradiated skin. In contrast, involvement of a direct photochemical process would make skin sensitivity well predicted by factors reflecting protection by absorption of the incident photons, that is, melanin content and thus phototype. Such observation is of importance for prevention and identification of sensitive individuals requiring extensive protection from UVA. This work aimed at addressing this issue by assessing the formation of CPDs upon UVA irradiation in the skin of two groups of volunteers with phototype either II or IV. In order to gather data on all possible bipyrimidine photoproducts, DNA damage was assessed by a liquid chromatography approach using mass spectrometry as detection (Douki *et al.*, 2000; Douki and Cadet, 2001). Comparison was carried out with the well-known effects of UVB and data were correlated with minimal erythemal doses (MEDs).

RESULTS

Erythemal response of phototype II and IV volunteers

A rather wide inter-individual variation of UVB-minimum erythemal dose (MED) determined with a solar simulator was observed within a same group of phototype (Table 1). In addition, some overlap in the UVB-MED was observed between the two groups. However, a significant (Student's $P<0.01$; Wilcoxon $P<0.015$) 1.6 time larger value for the UVB-MED was observed for phototype IV. The UVA-MED was difficult to accurately estimate for the phototype IV group because of a persistent darkening of the skin (Meirowski phenomenon) that hampered the visualization of the redness. Consequently, no significant difference was found between the UVA-MED of phototypes II and IV because of the poor quality of the readings for the latter group. Finally, no significant correlation was found between UVA-MED and UVB-MED neither in the phototype II nor IV group.

UVB-induced formation of dimeric photoproducts in skin biopsies

One 4 mm diameter biopsy was exposed for each volunteer to 0.2 J cm^{-2} UVB. DNA was then extracted and enzymatically hydrolyzed. The frequency of CPDs and 64PPs at the four bipyrimidine dinucleotides was then determined by HPLC associated with tandem mass spectrometry (Supplementary data, Supplementary Figure S1 online). First, the relative contribution of the different photoproducts was calculated for each volunteer and averaged according to the skin phototype. No difference was observed between the two groups (Figure 1a). As previously reported (Mouret *et al.*, 2006), TT CPD was the main photoproduct. TC CPD and TC 64 PP were next in abundance, whereas TT 64PP and CT CPD were produced in lower yield. CT 64PP, CC 64PP, and CC CPD were in amounts below the detection limit. Because the phototype did not affect the distribution, emphasis was then placed on the formation of TT CPD, the major UVB-induced pyrimidine dimer. The average level of TT CPD for the phototype II skin was 41.0 ± 10.5 TT CPD for 10^6 bases,

while it was 26.8 ± 7.3 for phototype IV. The respective medians were 39.7 and 26.9 TT CPD for 10^6 bases. This 1.5-fold difference was statistically significant (Student's $P<0.002$; Wilcoxon $P<0.01$).

Table 1. Minimal erythemal doses (MEDs) determined in the UVB and the UVA ranges for the different volunteers

Phototype II			Phototype IV		
Volunteer	UVB-MED	UVA-MED	Volunteer	UVB-MED	UVA-MED[1]
V3	1.30	30	V1	1.90	>50
V4	1.20	20	V2	1.00	30
V9	1.00	30	V7	0.80	30
V14	1.00	40	V8	1.20	20
V16	1.00	40	V10	2.98	50
V20	1.00	20	V11	1.20	30
V21	0.98	40	V12	2.40	40
V23	0.80	40	V13	1.90	30
V24	0.98	30	V15	1.50	30
V27	0.63	20	V17	1.50	50
V28	1.20	>50	V18	1.20	>50
V29	1.50	>50	V22	1.90	>50
Average[2]	1.05	35.8	Average[2]	1.62	40.8
SD	0.23	13.8	SD	0.63	14.4
Median	1.00	35.0	Median	1.63	35.0

Results are expressed in $J\,cm^{-2}$. The dose corresponds to the overall spectrum of the source. In the case of the solar simulator used for the determination of UVB-MED, UVB represented 5% of the incident energy. The UVA lamp emitted almost exclusively (0.003% UVB) in this wavelength range. Spectra are provided as supplementary information online.
[1]Evaluation of the UVA-MED for phototype IV skin is hampered by coloration due to the Meirowski phenomenon.
[2]For calculation of the average UVA-MED, a value of 60 Jcm^{-2} was arbitrarily chosen for individuals showing no erythema at 50.

UVA-induced formation of CPDs in skin biopsies

As we previously observed (Mouret *et al.*, 2006), UVA irradiation of skin (200 $J\,cm^{-2}$) led to the sole formation of TT, TC, and CT CPDs. When we compared the relative distribution of the different photoproducts no difference was observed between the two groups. TT CPD was largely predominant and represented 90% of the pyrimidine dimers for both phototype II and IV skins (Figure 1b). The average level of TT CPD was 39.6 ± 10.3 TT CPD for 10^6 bases for this first group, while it was 24.0 ± 7.5 TT CPD for 10^6 bases for the latter (Student's $P<0.001$; Wilcoxon $P<0.005$). Medians were 38.9 and 23.2 TT CPD for 10^6 bases, respectively. The frequency of TT CPD was similar in the DNA of biopsies exposed to either UVB or UVA (Supplementary data, Supplementary Figure S1 online). The same ratio between the two yields of TT CPDs was obtained for the phototypes II and IV: 1.05 ± 0.22 and 1.15 ± 0.25, respectively. It should be reminded that applied fluence was 1000-time larger for UVA than UVB and both resulted in the same level of photoproducts in DNA. The present value of the UVB/UVA ratio for the induction of CPDs is 3 times lower than our previous estimation (Mouret *et al.*, 2006), because a 295 nm filter was used for UVB irradiation in this study in order to block short wavelength photons.

Correlation between MED and formation of photoproducts

Correlation was searched between the MED and the induction of TT CPD. In the UVA range, no significant correlation could be found between the UVA-MED and the frequency of photoproducts in UVA-irradiated biopsies. This result is not surprising because UVA-MED was found to be difficult to determine for phototype IV skins. Limiting the analysis to phototype II did not yield a significant correlation either. In contrast, a significant correlation (Figure 2a) was observed between the UVB-MED and the level of TT CPD in UVB-irradiated biopsies ($r=-0.6253$, $P<0.002$). A good correlation ($r=-0.5386$, $P<0.01$) was also observed between UVB-MED and frequency of TT CPD in skin exposed

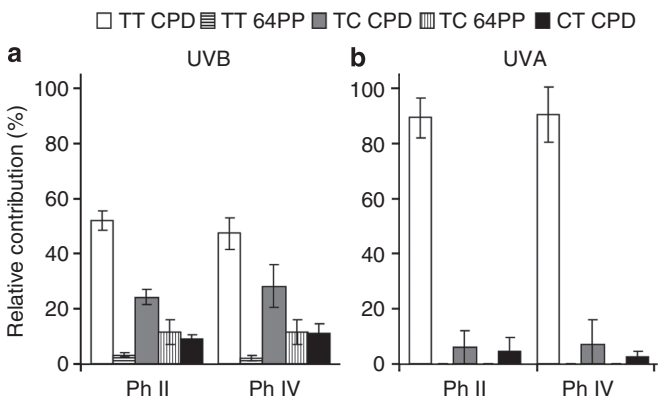

Figure 1. Relative distribution of dimeric photoproducts within skin biopsies of different phototypes. The samples were either exposed to (**a**) UVB or (**b**) UVA. Error bars represent the standard deviation ($n=12$). CPD, cyclobutane pyrimidine dimer; Ph, phototype; 64PP, pyrimidine (6–4) pyrimidone photoproduct.

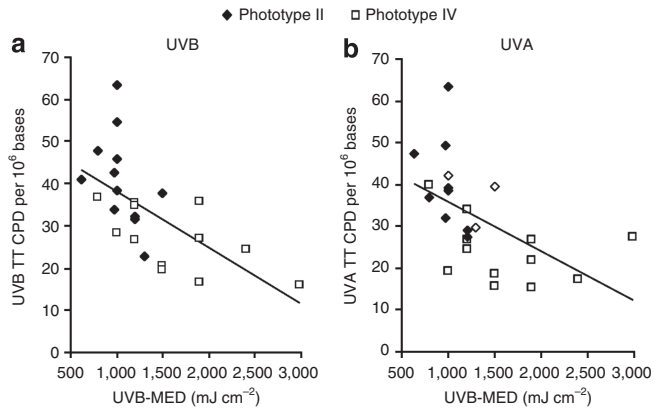

Figure 2. Correlation between the UVB-MED of volunteers and the yield of TT CPD in DNA. Biopsies were exposed to either (**a**) UVB (0.2 $J\,cm^{-2}$) or (**b**) UVA (200 $J\,cm^{-2}$). The MED values correspond to the overall spectrum of the solar simulator that emitted ~ 5% UVB. CPD, cyclobutane pyrimidine dimer; MED, minimum erythemal dose.

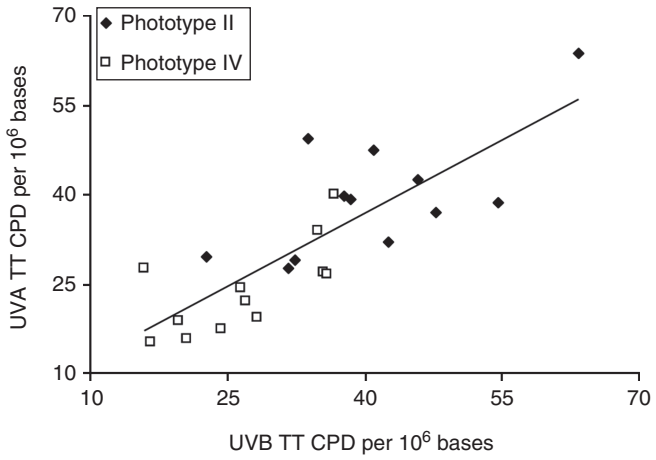

Figure 3. Correlation between the frequencies of TT CPD in the biopsies of volunteers exposed *ex vivo* to either UVB (0.2 J cm^{-2}) or UVA (200 J cm^{-2}) radiation. CPD, cyclobutane pyrimidine dimer.

to UVA (Figure 2b). Finally, a clear correlation was found between the yield of TT CPD in the UVB and the UVA range ($r=0.8075$, $P<0.001$; Figure 3).

DISCUSSION

This study addresses an important issue in the delineation of individual sensitivity toward the adverse effects of solar UV radiation. It is today clear that the risk of skin cancer is inversely related to constitutive pigmentation of the skin due to its protective effects against UV damage (Kaidbey *et al.*, 1979; Kollias *et al.*, 1991; Miyamura *et al.*, 2007). Indeed, a 70-fold higher risk factor for basal and squamous cell carcinomas was found in Caucasian Americans with respect to black or African-American skin (Lea *et al.*, 2007). The corresponding ratio was almost 20 for melanomas. As a likely explanation to this observation, it was estimated that darker skin afford a protection of about 13 times higher than light skins (Johnson *et al.*, 1998) and several articles showed the importance of constitutive skin pigmentation for the protection against UV-induced damage resulting from a single exposure to one MED (Tadokoro *et al.*, 2003, 2005; Del Bino *et al.*, 2006; Miyamura *et al.*, 2007). Interestingly, this trend does not seem to apply to pigmentation induced in skin by repeated exposures (Yamaguchi *et al.*, 2008). Therefore, it appears that constitutive pigmentation has a major role in individual skin UV sensitivity and particularly in the risk of skin cancer.

The Fitzpatrick's phototype classification based on self-reported sunburn sensitivity and tanning ability (Fitzpatrick, 1988) has been extensively used to accurately evaluate skin sensitivity, with the exception of Asian skins (Kawada, 1986). MED was proposed as another estimation of skin sensitivity to UV radiation. The two parameters were found to be correlated as shown in a French population (Amblard *et al.*, 1982). Gambichler *et al.* (2006) have also determined the relationship between skin phototypes and MED values for broadband UVB and found significant different mean UVB-MED values between the skin phototype classes. With these pieces of information in mind, we chose to compare the skin sensitivity as revealed by the phototype and the MED, with the extent of damage induced by UVA radiation within DNA. Data on the formation of UVB-induced DNA lesions, for which information is already available, were also gathered for comparative purposes. Twelve volunteers with phototype II and 12 with phototype IV were recruited and their MED was determined following exposure either to UVA or simulated sunlight. Skin biopsies were taken, irradiated *ex vivo* and DNA pyrimidine dimers were quantified therein using HPLC combined with mass spectrometry. It should be emphasized that the recruited volunteers were healthy individuals and that the results obtained in populations exhibiting abnormal extreme deficiency photosensitivity or DNA repair capacities might be different.

The MED determined with the solar simulator mostly reflects individual sensitivity to UVB, as erythemogenic properties of this UV range is at least two to three orders of magnitude higher than those of UVA. The MED determined with simulated sunlight will thus be referred to as UVB-MED. As previously reported, some overlap was observed between the two phototype groups for the UVB-MED. Nevertheless, a significant 1.6 larger value was obtained for phototypes IV than II, in agreement with published data. For instance in a wide study of the variation in sunburn sensitivity, Amblard *et al.* (1982) found a ratio of 1.8 between the UVB-MED for photoypes IV and II. Other authors reported values of 1.4 (McGregor *et al.*, 2002; Gambichler *et al.*, 2006) and 1.5 (Westerhof *et al.*, 1990). We also wanted to have access to a parameter reflecting UVA sensitivity of individuals. Interestingly, UVA exhibits small but significant erythemogenic properties, and determination of MED after exposure to UVA is routinely used to identify photosensitive patients (Kim and Lim, 1999). Therefore, we determined the MED following exposure to UVA, referred to as UVA-MED. The reading of the MED was much more tedious to analyze after UVA than after UVB exposure. Indeed, a strong Meirowski phenomenon, namely a photoinduced pigmentation of the skin, took place for phototypes IV, preventing an accurate determination of the UVA-MED. As a result, no difference was observed for the UVA-MED between the two groups. In the same way, Gambichler *et al.* (2006) found no correlation between UVA-MED and skin phototype. However, clear erythemal response could be seen for the vast majority of the phototype II volunteers, with a median UVA-MED of 26 J cm^{-2}, close to our determined value of 35 J cm^{-2}.

Like for UVB-MED, a significant difference was observed between the two groups for the induction of CPDs following *ex vivo* exposure of biopsies to 0.2 J cm^{-2} UVB, a biologically relevant dose corresponding for instance to those used in broadband UVB phototherapy. Interestingly, the ratio between the frequencies of TT CPD when the two skin types were compared (1.5) was identical to that of the UVB-MED (1.5). This similarity reflects the good correlation between the UVB-MED and the yield of TT-CPD produced by UVB radiation. It is thus expected that for different phototypes, exposure to one UVB-MED leads to the same amounts of DNA photoproducts, in contradiction with the report by

Tadokoro *et al*. (2003) that one UVB-MED induced more damage in fair skins than in dark ones. However, the UVB-MED of the volunteers in this last study ranged over a factor of more than 4 and included both highly resistant and highly sensitive individuals. In addition, a mild difference in the yield of damage for one UVB-MED was observed for groups with intermediate sensitivities, which correspond to those used in this study. Another confounding factor is the inclusion of several ethnic origins in the study by Tadokoro *et al*. (2003), while our groups were much more homogenous from that perspective.

The good correlation between UVB-MED and yield of CPD was also observed for skin biopsies after irradiation with 200 J cm^{-2} UVA, a dose received on a summer day and to twice the dose applied during a UVAI phototherapy treatment. The ratio between the frequency of CPDs in phototype II and IV skins was 1.6 (II vs. IV), close to the value of 1.5 (IV vs. II) for the ratio of UVB-MED. It must be emphasized that the relative distribution of dimeric lesions was different from that observed with UVB, in agreement with previous observations (Douki *et al*., 2000, 2003; Courdavault *et al*., 2004; Mouret *et al*., 2006). These results show that both the phototype and the UVB-MED efficiently predict the sensitivity of the skin to the induction of TT CPD not only after exposure to UVB but also to UVA. Such a correlation was reported before for UVB-induced CPDs (Young *et al*., 1998a, 2000; Del Bino *et al*., 2006), but to our knowledge this is previously unreported for UVA.

In view of these results, it could be tempting to propose UVB-MED as a reliable predictor of DNA damage in the estimation of the protection afforded by sunscreens in the UVA range. This proposal, that could somewhat be justified in the UVB range where both erythema and DNA damage induction exhibit a maximum, is however misleading. A first drawback in such an approach for UVA is the complete lack of correlation between the erythemal response and the yield of CPDs, as observed in phototype II volunteers exposed to UVA. It must also be emphasized that sunscreens exhibit a much higher protection in the UVB than the UVA range. Application of the sunscreen, particularly if it has a very high sun protection factor, to the tested area leads to an increase in the UVA to UVB ratio of the radiation reaching skin cells, thereby completely modifying the respective induction of erythema and dimers. A true evaluation of the genetic protection afforded by sunscreens toward DNA would thus be the direct quantification of the CPDs. Work is in progress in our group to further establish this approach (Mouret *et al*., 2011).

Our results also provide some information on the origin of CPDs in the UVA range. An important issue is whether these photoproducts arise from a direct mechanism or a photosensitized reaction. Our present observations do not allow us to draw definitive conclusion, but permit to rule out some hypotheses. In particular, no correlation could have been observed between the yields of UVA- and UVB-induced CPDs if the former photoreaction involved endogenous photosensitizers present in larger amount in dark skins. In addition, our data show that the key parameter for the UVA-induced formation of CPD is the physical dose of light reaching DNA. This important observation made under physiologically relevant conditions is another suggestion of a direct photoreactivity of DNA exposed to UVA, even in whole skin. It should be reminded that experiments have shown that UVA irradiation of naked DNA, namely in the absence of photosensitizers, gave rise to CPDs (Quaite *et al*., 1992; Zhang *et al*., 1997; Kuluncsics *et al*., 1999; Jiang *et al*., 2009; Mouret *et al*., 2010). In addition, the yield of CPD is similar with that observed in cultured cells, thus showing that no endogenous photosensitizer enhances the efficiency of this photoreaction (Kuluncsics *et al*., 1999; Perdiz *et al*., 2000; Mouret *et al*., 2010). Yet, the distribution of photoproducts upon UVA irradiation is drastically different from that after exposure to UVB. This result points to a need in the identification of the initial photophysical events.

The data suggesting a direct photochemical pathway in the UVA induction of CPDs emphasize the need to consider the UVA range in the estimation of cancer risk. Indeed, DNA appears now as intrinsically sensitive to this class of radiation, with no complete protection possibly afforded by exogenous components such as antioxidant orally supplemented. The only complete protection against UVA appears to be sun avoidance or complete blocking of radiation. Our results also bring additional evidence for the lack of innocuousness of artificial UVA-rich sources used in tanning equipments. Data on UVA-MED obtained with phototype II individuals show that erythema cannot be expected to act as a warning signal before occurrence of significant DNA damage with UVA-rich sunbeds. It thus appears that identification of the most sensitive individuals toward UVA induction of CPDs before exposure to either natural or artificial UV is a key aspect of prevention. Fortunately, we showed that the determination of the classical UVB-MED and more conveniently of the skin phototype are useful tools in the prevention of adverse effects of UVA toward DNA, as they are for UVB.

MATERIALS AND METHODS

Panel of volunteers

Twenty-four healthy volunteers were recruited through advertising in print media and by word of mouth to participate. Volunteers had to be healthy men aged between 20 and 33 years. Twelve exhibited skin phototype II and 12 skin phototype IV according to Fitzpatrick's skin typing system based on self-reported erythema sensitivity and tanning ability (type II, burns easily and tans with difficulty and type IV, burns occasionally, tans readily; Fitzpatrick, 1988). A visual evaluation of skin color was also made to confirm the skin phototype. Exclusion criteria included smoking, medical treatment, abnormal photosensitivity, recreational sunbathing, and exposure of back skin to sunlight or artificial source UV within 3 months of the start of the study, evolutive dermatosis of the back, presence of naevi in the irradiation areas. The study was carried out with the approval of the local committee for the protection of persons, and the volunteers signed an informed consent. The experiment was conducted according to the Declaration of Helsinki Principles guidelines.

MED determination

MEDs induced by either simulated solar radiation or UVA were determined after irradiation on the back of each volunteer. First,

the MED was determined using a solar simulator equipped with a xenon lamp (Dermolum UM-W*, Muller, Moosinning, Germany). The fluence rate was determined by a wide-band thermopile radiometer covering the entire emission spectrum, from UV to infrared radiation. The content of the lamp in UVB to the overall spectrum was ~5%. Thus, reported values for MED correspond to energy of the full spectrum and should be divided by a factor of 20 to be expressed in UVB similar to some other works. The erythemal response after irradiation induced by this type of equipment mostly reflected the UVB effect and was thus referred to as UVB-MED. The minimal dose of solar-simulated radiation required to induce a just perceptible erythema at 24 hours (UVB-MED) was determined by geometric exposure series (Diffey and Farr, 1989) at nine sites from 0.4 to 2.384 J cm^{-2} for skin phototype II and from 0.5 to 2.980 J cm^{-2} for skin phototype IV, using a commercial template with a series of 1.5 × 1.5 cm openings with removable flaps. A high-pressure UVA metal halide lamp (UVA 700 L Waldmann, Villingen-Schweinningen, Germany; Supplementary data, Figure S2 online) was used to determine the MED induced by UVA (UVA-MED). The proportion of UVB was 0.003%, with intensity at 320 nm representing 0.04% of that at 365 nm. This distribution weighted by the Commission Internationale de l'Eclairage erythema action spectrum yielded a spectrum centered in the UVA range (Supplementary Figure S3 online). The fluence rate was determined by using the built-in probe of the irradiator as described by the manufacturer. For the determination of UVA-MED exposures of 10, 20, 30, 40, and 50 J cm^{-2} on an area of 2 × 2 cm were applied to all the volunteers. For two phototype II and three phototype IV volunteers, the applied UVA dose was limited to 50 J m^{-2} even in the absence of erythemal response. For both solar-simulated radiation and UVA, readings were performed 24 hours after irradiation. A late reading 48 hours after irradiation was also made after UVA irradiation to put out the immediate darkening for the skin phototype IV.

Irradiation of skin biopsies

Four 4 mm punch biopsies were taken for each volunteer from an unexposed area of the skin (top of the buttock). A stitch was made and the wound was controlled after 24 hours. The biopsies were kept in fresh media for no longer than 90 minutes. They were then rinsed four times in phosphate-buffered saline, placed in 35 mm Petri dishes with phosphate-buffered saline. Two biopsies were irradiated with UVA, one with UVB and the last was kept as an untreated control. UVA irradiations were performed on ice with 700 W UVA lamp (UVA 700 L, Waldmann) described above. The overall fluence was 200 J cm^{-2} (irradiation time 45 minutes). UVB irradiation, carried out at room temperature, involved the use of a VL 215 G irradiator (Bioblock Scientific, Illkirch, France) fitted with two 15 W tubes with a broad-spectrum distribution with a maximum at 312 nm. A 295 nm cut-off filter (WG295, Schott, Yverdon les Bains, Switzerland) was placed on top of the samples in order to remove the minute component of high-energy radiation. The proportion of UVC was thus limited to 0.07% with respect to UVB (Supplementary data, Figure S4 online). The fluence rate was measured by a VLX 3W radiometer (Vilbert Lourmat, Marne la Vallée, France) equipped with a 312 nm probe. The applied fluence was 0.2 J cm^{-2}, corresponding to irradiation periods of 4 minutes. After irradiation, biopsies were kept frozen at −80 °C before extraction in order to prevent DNA repair.

Quantification of DNA photoproducts

For DNA extraction, human skin was first grinded in liquid nitrogen. For the UVA samples, two biopsies were combined before extraction. Then, DNA was extracted with the Qiagen DNEasy Tissue Kit (Courtabœuf, France). The powder obtained after cold grinding was recovered in the first lysis buffer prior to be incubated overnight at 55 °C with proteinase K. An RNase-A treatment and a second lysis step (buffer AL) were performed before loading the samples onto the DNEasy mini spin column. DNA was then eluted in two successive steps by using 200 µl of water. The sample was freeze-dried overnight and the resulting DNA residue was dissolved in 50 µl of 0.1 mM deferroxiamine mesylate solution. The solution was then incubated in a first step (2 hours, 37 °C) at pH 6 in the presence of nuclease P1, DNase II, and phosphodiesterase II. The pH was then raised to 8 by the addition of Tris. A second incubation period in the presence of phosphodiesterase I and alkaline phosphatase (37 °C, 2 hours) yielded digested DNA with normal bases as nucleosides and photoproducts as dinucleoside monophosphates. Samples were then injected on an HPLC systems (Agilent, Massy, France) connected to a reverse-phase HPLC column (150 × 2 mm ID, 5 µm particle size, ODB, Montluçon, France). The detection was provided first by a UV detector aimed at quantifying normal nucleosides. Photoproducts were detected by a tandem mass spectrometer (API 3000, SCIEX, Thornill, Canada) used in the reaction-monitoring mode as previously described (Douki *et al.*, 2000; Douki and Cadet, 2001). The four CPDs and the four 64PPs (TT, TC, CT, and CC derivatives) were quantified individually in the same HPLC run. Results were expressed in number of lesions per million normal bases.

Statistical analyses

For comparison of averages between the two phototype groups, a Student's test was used after a Fisher test of the identity of variance. Data were also analyzed by calculating the median that was found to be close to the average (with the exception of UVA-MED), and by using the non-parametric Wilcoxon matched pair test. For trends comparisons, the Pearson's coefficient was calculated. It was then used to determine the *t*-value that was used for Student's test with *n*-2 degrees of freedom. Only differences with *P*-values <0.05 were considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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