Repeated Ultraviolet Exposure Affords the Same Protection Against DNA Photodamage and Erythema in Human Skin Types II and IV but is Associated with Faster DNA Repair in Skin Type IV

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We have investigated the photoprotective properties of induced pigmentation using erythema and epidermal DNA photodamage as endpoints. Previously unexposed buttock skin of 12 young, healthy adults (six skin type II and six skin type IV) was exposed daily (Monday to Friday) for 2 wk (days 1–12) with 0.65 minimal erythema dose of solar simulated radiation. Mean skin type IV minimal erythema dose was 1.8-fold greater than for skin type II. Compared to skin type II, solar simulated radiation treatments produced less erythema and more tanning in skin type IV. To assess DNA photodamage, biopsies were taken and prepared for paraffin sections that were stained with a monoclonal antibody for thymine dimers. Thymine dimers were quantified by image analysis. The single exposure data (0.65 and 2 minimal erythema dose) showed that DNA damage was related to physical dose (J per cm²) independent of skin type. Our data also showed that DNA photodamage accumulates in both skin types with repeated, suberythema doses of solar simulated radiation. On day 12, there were more thymine dimers in skin type IV than skin type II, again indicating that physical rather than biologic dose determines the level of DNA damage. Comparisons on days 12 and 19, however, showed a much greater loss of thymine dimers in skin type IV, suggesting better thymine dimer repair. Protection factors for erythema and thymine dimers were calculated and shown to be about 2 in both skin types. This provides further indirect evidence that DNA is a chromophore for erythema, but also suggests that a tan may not be the major factor in natural photoprotection. Key words: melanogenesis/protection factor/thymine dimers. J Invest Dermatol 118:825–829, 2002

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Abbreviations: PF, protection factor; SSR, solar simulated radiation; T=T, thymine dimers.
indirect measurement of DNA damage (unscheduled DNA synthesis) or directly using a monoclonal antibody to thymine dimers (T=T). There was good correlation between SSR dose and the levels of T=T in epidermal nuclei and the levels of T=T also correlated well with the levels of unscheduled DNA synthesis. In this study we have investigated the photoprotective effects of tanning induced by repeated daily suberythemal SSR exposure. These tanning treatments were carried out over a 2 wk period, which may be considered as a "simulated beach holiday exposure" during which, for example, sunscreens may have been used to minimize sunburn. We have selected skin types II and IV and the photoprotection endpoints we have used are the MED and DNA damage (T=T, a class of cyclobutane pyrimidine dimer). These studies have enabled us to determine PF that are analogous to the widely used sun protection factors (SPF) used to classify the photoprotective properties on sunscreens.

We have shown in unpublished pilot studies that DNA photodamage accumulates in skin types II and III with repeated, suberythemal doses of SSR. The accumulation of DNA photodamage at suberythemal UVB exposures has also been shown in uninvolved human skin of psoriatics undergoing phototherapy (Bataille et al, 2000). This is an important factor to consider as the accumulation of unrepairable DNA damage may have serious implications for skin cancer risk.

In this study we have investigated two factors concerned with the tanning response in different human skin types: first, the level of protection a tan offers against erythema and DNA photodamage, and second the accumulation and repair of DNA damage in the acquisition of a tan.

MATERIALS AND METHODS

Volunteers The local ethics committee approved the study. Twelve healthy young people were selected on the basis of their constitutive skin color and a questionnaire on their responses to solar exposure. According to the Fitzpatrick classification (Fitzpatrick, 1988) six volunteers were skin type II (burns easily, tans with difficulty) and six were skin type IV (tans well, burns rarely). Mean age was 24 y ± 7 (SD). Eight volunteers were male and four were female. All volunteers gave informed consent.

UVR source and dosimetry SSR was obtained from a 1000 W xenon arc solar simulator (Oriel, U.K.). The spectrum of this source has been previously published (Sheehan et al, 1998). The irradiance over the 295–400 nm range was determined with a double-monochromator spectroradiometer (Benthem Instruments, Reading, U.K.) that had been calibrated against a deuterium source measured by the National Physics Laboratory (Tedddington, Middlesex, U.K.). These data were used to calibrate a wide-band thermopile radiometer (Medical Physics, Dryburn Hospital, Durham, U.K.), which was used for routine calculation of exposure dose (295–400 nm) expressed as J cm⁻². Multiplication of the SSR and U.K. (51°N) solar spectra with the Commission Internationale de L’Eclairage (CIE) erythema action spectrum (McKinlay and Diffey, 1987) shows that 92% and 81%, respectively, of the effective erythema energy is UVB (280–320 nm).

Study protocol All exposures were given to previously unexposed buttock skin. The 24 h just perceptible MED was determined by a geometric exposure series of eight (1 cm x 1 cm) sites using a 25% incremental dose series. Duplicate 6 x 5 cm sites were exposed daily (Monday to Friday) for two consecutive weeks (days 1–12) with 0.65 MED or T=T and MED readings from exposed and nonexposed sites as well as biopsies from exposed and nonexposed sites were taken as described. In each case the ratio of MED or T=T level on pre-exposed sites to MED or T=T on nonexposed sites was calculated and expressed as the PF. The PF represents an increase in protection against SSR-induced erythema or DNA photodamage following repeat, suberythemal exposure. The PF was calculated using the same approach as that for the determination of SPF. Thus, per person, the PF for erythema and DNA damage on the chronically exposed sites was determined using the expression PF = (MED or T=T value on treated site)/(MED or T=T value on a nontreated site).

In each case, mean and SD were calculated for skin type II and skin type IV separately. Single paired t tests (two-tailed) were carried out to compare values from exposed and nonexposed sites.

RESULTS

The tanning treatment results in more erythema in skin type II and more tanning in skin type IV. At the end of week 3 (day 19), the daily tanning treatment resulted in a light tan in skin types II and a light–moderate tan in skin types IV. The differences in quantitative data reflect these semiquantitative observations. The onset and duration of erythema and tanning in response to SSR treatment are shown in Figs 1(a) and 1(b), respectively. All readings were background corrected and so are relative to proximal, non-SSR–exposed sites. The degree of tanning was greater in skin type IV. In skin type II, there is a cumulative erythema peak on day 5, which matched observations by eye. The erythema is less prominent by day 12 and was not perceptible to the naked eye by day 19. In all cases, skin type IV showed a similar pattern but with lower levels of erythema and more evident tanning.

With both skin types there is a steady increase in melanin content as the study progressed. With skin type II volunteers, however, the melanin appears to decrease (not statistically significant, p = 0.38) during the first week and steadily rises during the second week. We have previously reported this in humans and mice (Kipp et al, 1998; Sheehan et al, 1998). The reasons for this are not known.

DNA damage is related to physical SSR dose when administered as a single exposure or as repeat exposures Figure 2 shows that DNA damage increases with increasing SSR dose. Figure 2(a) shows the epidermal DNA damage induced by single exposures of 0.65 MED or 2 MED SSR in skin types II and IV. When biopsies are taken immediately after exposure, the data indicate that the amount of epidermal DNA damage per single biologic dose (0.65 MED or 2 MED) is related to the physical dose. Figure 2(b) shows the epidermal DNA damage induced by repeat
exposures of 0.65 MED SSR in skin types II and IV when biopsies were taken immediately after the last tanning treatment dose. Cumulatively, the two skin type groups got the same biologic dose (10 daily exposures of 0.65 MED), but the data show that the higher amount of DNA damage in skin type IV appears to be related to higher physical dose.

Epidermal DNA damage is more persistent in skin type II than in skin type IV. Figure 3 shows the levels of epidermal DNA photodamage immediately after the last tanning treatment and the levels of damage in those tanned sites 1 wk later. Figure 3(a) shows means of each skin type group. Figure 3(b) shows the individual volunteer data. In skin types IV, there was a significant reduction (68.9%) in the amount of DNA damage in the tanned sites after 1 wk (p = 0.02). Skin type II displayed a smaller reduction (37.3%) in the amount of DNA damage after 1 wk but this was not significant (p = 0.18). Skin type IV had higher levels of DNA damage induced by the tanning protocols than skin type II (although this was not significant, p = 0.1) but generally repaired the damage better over the period of 1 wk.

The PF for DNA photodamage and erythema are the same and are independent of skin type. Skin type IV showed slightly higher PF than skin type II for both erythema and DNA photodamage but the difference was not significant (p = 0.3 for erythema and p = 0.23 for DNA). The PF data for erythema and DNA damage are shown in Fig 4. The initially assessed mean MED value did not change on the second assessment on untreated skin. There was no significant difference between the PF for DNA and erythema in skin type II or IV (p = 0.99 and p = 0.83, respectively).

DISCUSSION

We have assessed the photoprotective properties of induced tanning in skin types II and IV using erythema and T=T as endpoints. Our protocol simulated “sensible tanning” with daily suberythemal exposure to SSR and was designed to minimize the effects of erythema accumulating after a few exposures. During the SSR treatment there was a clear difference in response between the two skin types. As expected, skin type II showed more erythema than skin types IV who in turn showed a better tanning response. One week after the last tanning treatment, SSR-exposed and nontreated sites were challenged with 2 MED SSR and PF were determined for erythema and T=T. The results (Fig 4) showed that in all cases the PF were in the region of 2±3. The values were slightly higher in skin type IV but the differences were not significant. The similarity of the PF against erythema and T=T supports the hypothesis that DNA is a major chromophore for erythema as suggested by Young et al (1998, 2000). The PF of about 2 against erythema confirm our earlier studies in skin types II and III (Sheehan et al, 1998). The lack of difference between skin types II and IV suggests that the protection afforded by the treatment was not dependent on the level of tan as there was, as expected, a marked difference in the tanning response of the two skin types. It has been reported that UVR-induced stratum corneum thickening is important in photoprotection (Kaidbey and Kligman, 1978; Gniadecka et al, 1996). We did not assess stratum corneum thickening in this study, but we have previously shown no skin-type-dependent difference in the number of stratum corneum layers after repeated daily exposure to suberythemal SSR.
and III (Sheehan et al, 1996) physical dose (50 J per cm² MED) but the skin type IV group received a higher cumulative doses of SSR. Furthermore, we have previously shown that the erythemal response to repeated suberythemal SSR is greater and more persistent in skin type II than in skin type III (Sheehan et al, 1998) and in this study we have seen the same trend between skin type II and IV. It has been suggested that DNA damage stimulates pigmentation through upregulation of tyrosinase levels and that tanning is part of the eukaryotic SOS response (Eller et al, 1997). Our data suggest that this response may only occur in skin types that tan well and that this may be the reason that they are more resistant to skin cancer rather than the protective properties of a tan per se.

We have previously shown that the erythemal response to repeated suberythemal exposures may be more relevant to risk assessment than a single exposure response as the former is more relevant to a real-life situation. We believe that DNA is the chromophore for erythema (Young et al, 1998), which would mean that erythema is the consequence of DNA photolesions such as cyclobutane pyrimidine dimers. It is possible that the persistence of erythema in skin types I (Young et al, 1991) and II is due to the lack of DNA repair in these skin types compared with skin types III and IV. Here, it is interesting to note that skin-cancer-prone xeroderma pigmentosum patients who lack DNA repair capacity also show persistent erythema (Berg et al, 1998).

The widespread and popular belief that a tan is photoprotective is often used to justify tanning. Our study shows that the protection afforded by repeat low dose SSR exposure is modest and does not seem to be related to tanning per se. Using tanning protocols with higher daily doses from a different SSR source, we have previously reported a lack of any protection from SSR-induced epidermal DNA damage in skin types I and II but PF of about 2 in skin types III and IV (Young et al, 1991; Potten et al, 1993). The low level of photoprotection that we have observed suggests that any photoprotective benefit afforded by a tan is outweighed by the photodamage incurred in its acquisition, especially in skin type II. It should be remembered, however, that our study sites had not previously been exposed to UVR. More constant exposure, along with time, may alter any risk-benefit assessment. A PF of 2 may be considerable over a lifetime, reducing effective UVR dose by 50%, if the tan can be maintained with lower levels of solar exposure, especially in white skin types that tan well. The stimulation of exposure has induced DNA repair in skin type IV but not skin type II.

This proposed greater T=T repair in skin type IV is in accordance with the hypothesis of Gilchrest and Eller (1999) whose data suggest that DNA excision fragments or DNA repair intermediates are a trigger for melanogenesis. It has been suggested that DNA damage stimulates pigmentation through upregulation of tyrosinase levels and that tanning is part of the eukaryotic SOS response (Eller et al, 1997). Our data suggest that this response may only occur in skin types that tan well and that this may be the reason that they are more resistant to skin cancer rather than the protective properties of a tan per se.

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DNA repair by regular low level UVR exposure may be an important factor to consider in risk assessment and public health campaigns. The cumulative DNA damage incurred on acquiring and maintaining a tan, however, remains an important risk and must be considered very carefully.

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