# Protection by Ultraviolet A and B Sunscreens Against *In Situ* Dipyrimidine Photolesions in Human Epidermis is Comparable to Protection Against Sunburn

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**Sunscreens prevent sunburn and may also prevent skin cancer by protecting from ultraviolet-induced DNA damage. We assessed the ability of two sunscreens, with different spectral profiles, to inhibit DNA photodamage in human epidermis *in situ*. One formulation contained the established ultraviolet B filter octyl methoxycinnamate, whereas the other contained terephthalylidene dicamphor sulfonic acid, a new ultraviolet A filter. Both formulations had sun protection factors of 4 when assessed with solar simulating radiation in volunteers of skin type I/II. We tested the hypothesis that sun protection factors would indicate the level of protection against DNA photodamage. Thus, we exposed sunscreen-treated sites to four times the minimal erythema dose of solar simulating radiation, whereas vehicle and control sites were exposed to one minimal erythema dose. We used monoclonal antibodies against thymine dimers and 6–4 photoproducts and image analysis to quantify DNA damage in skin sections. A dose of four times the minimal erythema dose, with either sunscreen, resulted in comparable levels of thymine dimers and 6–4 photoproducts to one minimal erythema dose ± vehicle, providing evidence that the DNA protection factor is comparable to the sun protection factor. The lack of difference between the sunscreens indicates similar action spectra for erythema and DNA photodamage and that erythema is a clinical surrogate for DNA photodamage that may lead to skin cancer. *Key words: DNA photodamage/photoprotection/skin cancer/sunscreen/UVA protection. J Invest Dermatol 115:37–41, 2000***

Sunscreens are formulated to prevent sunburn. Their efficacy is indicated by their sun protection factor (SPF), which is evaluated, according to strict industry guidelines, using solar simulating radiation (SSR). Sunscreen use is widely advocated to inhibit the long-term effects of solar exposure, such as skin cancer and photoageing, and animal studies have supported this approach (Harrison *et al*, 1991; Fourtanier, 1996). Some studies in humans have shown that sunscreen use is associated with a reduction in actinic keratoses (Thompson *et al*, 1993; Naylor *et al*, 1995) that are widely regarded as precursors for squamous cell carcinomas (SCC). More recently, in a 4.5 y randomized controlled study with a broad spectrum SPF 16 product, Green *et al* (1999) showed a 40% reduction in the total number of SCC, but no effect on the number of people with SCC nor on basal cell carcinoma, whether assessed by total count or number of people with tumors. Other studies have suggested a positive correlation between sunscreen use and malignant melanoma, most recently the work of Autier *et al* (1998). It has been suggested that ultraviolet A (UVA) may play a role in malignant melanoma (Setlow and Woodhead, 1994). Thus, one reason for a possible association between sunscreen use and malignant melanoma may have been the lack of adequate UVA (320–400 nm) protection in sunscreens until quite recently. Some workers, based entirely on *in vitro* studies, have suggested that sunscreen filters or pigments themselves are photomutagenic (Knowland *et al*, 1993; Dunford *et al*, 1997) and therefore potentially carcinogenic.

In reality, the role of sunscreens in the prevention of skin cancer is poorly understood, but has important public health implications (McGregor and Young, 1996), especially in education campaigns about their value in reducing the high incidence of skin cancer in susceptible white-skinned populations who sunburn easily. Ideally, the role of sunscreens in the prevention of skin cancer should be assessed by prospective randomized case control studies as done by Green *et al* (1999). In practice such long-term (at least 4-7 y) studies present practical and ethical problems. Another approach is to use short-term surrogates for skin cancer. For example, there is increasing evidence that nonmelanoma human skin cancer involves at least two processes: (i) UVR-induced dipyrimidine DNA lesions, such as cyclobutane pyrimidine dimers (CPDs), and consequent mutation of the p53 gene (Brash *et al*, 1996), and (ii) UVR-induced immunosuppression (Nishigori *et al*, 1996a), which may be mediated via CPDs (Kripke *et al*, 1992). Mouse studies have shown that sunscreens can inhibit DNA photodamage (Wolf *et al*, 1993; Ley and Fourtanier, 1997) and p53 mutation (Ananthaswamy *et al*, 1997). Surprisingly, there are very few quantitative data on the ability of sunscreens to prevent DNA photodamage in human skin *in vivo,* and no study to date has adequately assessed the relationship

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Abbreviations: (6–4)PP, 6–4 photoproduct; CPD, cyclobutane pyrimidine dimer; SSR, solar simulating radiation; SPF, sun protection factor; TT, thymine dimer.

between SPF and degree of protection from DNA photodamage in human epidermis. A recent study (Bykov *et al*, 1998) reported that protection from UVB (290–320 nm)-induced DNA photodamage was at least as good as labeled SPF as determined by the manufacturer. SPF, by definition, however, is assessed with SSR and is valid only as an indicator of protection from erythema by a given SSR source. UVB-rich sources, including SSR sources, can result in a 100% overestimation of SPF when compared with summer sunlight (Farr and Diffey, 1985; Sayre *et al*, 1994). Thus, the protection factor for erythema against a UVB-rich source is likely to have been very much higher than the SPF stated on the product tested, so that a comparison between labeled SPF and protection from UVB-induced DNA photodamage is not valid. An earlier study also used a UVB source and did not estimate DNA protection factors (van Praag *et al*, 1993). Studies on the ability of sunscreens to inhibit UVR-induced immunosuppression have resulted in mixed conclusions (see Young and Walker, 1995 for review) with several authors reporting that sunscreens do not afford immunoprotection.

A very important question relating to the use of sunscreens to inhibit skin cancer is whether SPF is indicative of the level of protection against skin cancer or its surrogates. The answer to part of this question was one of the goals of this study, which was designed to determine whether SPF is indicative of the level of protection against one class of CPD, the thymine dimer (TT), and 6-4 photoproducts ((6-4)PP) induced by SSR in human epidermis *in vivo*. We also set out to determine if erythema *per se* is indicative of DNA photodamage, especially as we have recently reported that the action spectra for human erythema and TT in human epidermis *in situ* are very similar (Young *et al*, 1998a), which suggests that DNA is a chromophore for erythema. We approached this problem by comparing the DNA protective abilities of two sunscreens, each with the same SPF (as confirmed in our laboratory) but with quite different absorption spectra. The same SSR source was used for the SPF determination and the DNA studies. As photobiological effects are very wavelength dependent, we believe that it is essential to use the same SSR source when comparing protection from erythema, i.e., SPF, and other endpoints. The significance of this point is often not appreciated and this has resulted in considerable confusion in the photoprotection literature.

## MATERIALS AND METHODS

**UVR source** The UVR source was SSR from an Oriel (Leatherhead, U.K.) Solar Simulator equipped with a 1 mm WG320 filter (Schott, Mainz, Germany), a 1 mm UG11 (No.18812, Schott, Clichy, France), and a dichroic mirror. The emission spectrum is shown in **Fig 1**. SSR doses were routinely monitored with a broad band thermopile radiometer (Medical Physics, Dryburn Hospital, Durham, U.K.) and calculated by making comparisons with spectroradiometric determinations (Bentham Instruments, Reading, U.K.).

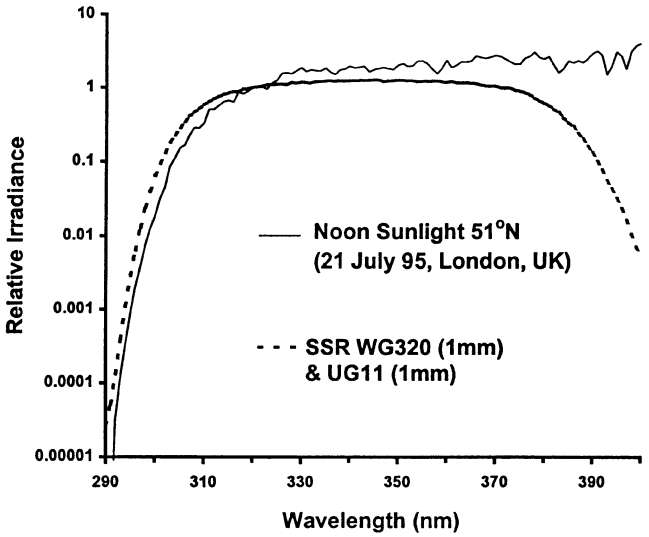


**Figure 1. Emission spectrum of SSR source compared with London U.K. noon summer sunlight.** The xenon source was modified by a dichroic mirror and filtered with two Schott filters (WG320, 1 mm; UG11, 1 mm). The data are normalized at 320 nm.

**Volunteers** The study was approved by the Ethics Committee of St. Thomas's Hospital, London. Volunteers gave informed consent and were selected according to the following criteria: inclusion - aged 18–45, skin types I or II; exclusion - pregnancy, a history of nude tanning whether by sunlight or sunbeds, any medication within 7 d prior to the start of the study with the exception of oral contraception, any investigational drug within 28 d of the start of the study. The 24 h just perceptible minimal erythema dose (MED) for 14 volunteers (skin type II with one skin type I) was assessed clinically on previously unexposed buttock skin sites (1 cm × 1 cm) using a geometric series of six exposure doses with increments of $\sqrt{2}$. Eight volunteers with the same MED (2.0 J per $cm^2$ – full SSR UV spectrum) and one whose MED was 2.8 J per $cm^2$ were selected from this group and used for the SPF part of the study. Of these, eight agreed to take part in the study to assess the ability of sunscreens to protect from DNA photodamage. In all cases, erythema was also assessed in triplicate by a reflectance device (Dia-Stron, Andover, U.K.). The erythema index per experimental site is ''background corrected'' by subtracting the erythema reading from an adjacent nonirradiated control site.

**Sunscreens and vehicle** Two coded sunscreen formulations and their common uncoded vehicle were provided by L'Oréal Recherche, Clichy, France. One formulation contained octyl methoxycinnamate, a widely used UVB absorber ($\lambda_{max} = 308$ nm), whereas the other contained terephthalylidene dicamphor sulfonic acid, a broad spectrum UVA filter with maximum absorption at 345 nm. The absorption profiles of the sunscreens and their common vehicle are shown in **Fig 2(*a*)**. We color coded the preparations to minimize the chance of any application errors. All samples were stored at room temperature. The formulations were returned to the supplier after the study for re-analysis of their active ingredients, and their stability during the study period was confirmed.

**SPF determination** COLIPA test method guidelines were followed for *in vivo* SPF determination (except that we used $\sqrt{2}$ SSR dose increments rather than 25%), and we used a demarcated 42 $cm^2$ area (6 cm × 7 cm) of previously unexposed buttock skin of nine volunteers. The product was applied at a rate of $2.00 \pm 0.04$ mg per $cm^2$. Therefore each application was approximately 84 mg per 42 $cm^2$. All topical applications took place 15–20 min before irradiation. All applications were determined by weighing and were ''spotted'' evenly around the demarcated area and smoothed, using a finger cot, over the surface of the skin as evenly as possible (including peripheral areas). SSR exposure areas were demarcated with 1 cm × 1 cm templates. The SPF was calculated according to the expression (MED with sunscreen)/(MED without sunscreen). *In vitro* assessment of monochromatic protection factors were made according to the method of Diffey and Robson (1989).

**Experimental design of DNA photoprotection studies** Ideally, the study should have been designed using the same approach as for SPF assessment. SSR dose–response studies with and without sunscreen on the same person were not possible, however, because of a limit of five biopsies imposed by the Ethics Committee. Instead, the studies were designed to test the assumption that SPF would be indicative of the level of protection against DNA photodamage. In other words, does n × MED with a sunscreen of SPF = n give the same level of DNA damage as 1 MED without sunscreen? SPF evaluation had shown that both formulations had an SPF of 4 (see *Results* for full details). The sunscreen-treated buttock sites (same application techniques and operator as described above) were exposed to 4 MEDs SSR about 20 min after application. The vehicle and nontopically treated sites were exposed to 1 MED. Immediately after exposure, 4 mm punch biopsies were taken, under local anesthesia, from all four sites plus one nonirradiated nontopically treated control site.

**Assessment of TT and (6–4)PP** Biopsies, taken immediately after SSR exposure, were processed for analysis of TT and (6–4)PP. The details have been published elsewhere (Chadwick *et al*, 1995; Young *et al*, 1996). In summary, 3 μm paraffin sections were cut and immunostained with a monoclonal antibody for TT (TDM-1) (Mizuno *et al*, 1991), or for (6–4)PP (64 M) (Mori *et al*, 1991), followed by a DAB-peroxidase immune reaction that gives brown nuclear coloration. Nuclei were counterstained

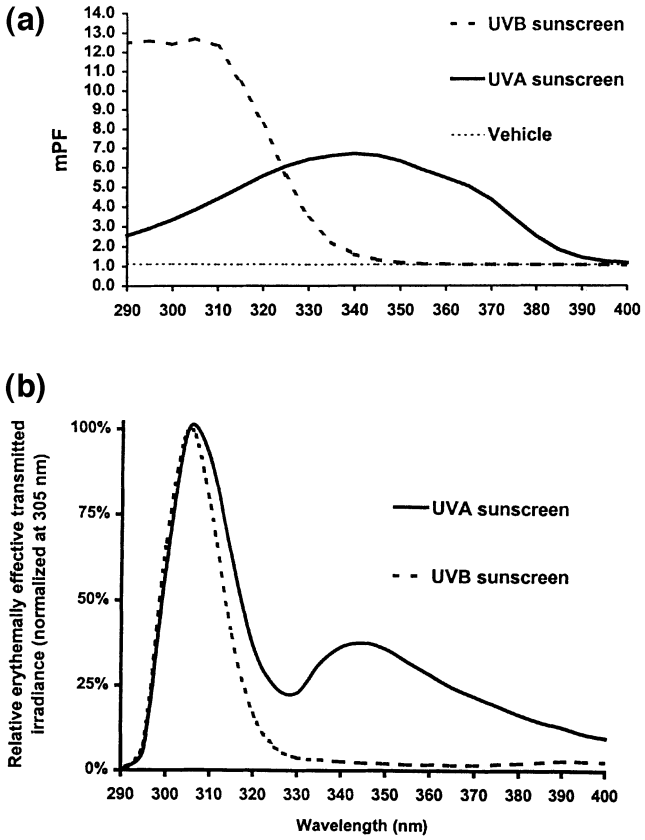


**Figure 2. Equivalent protection against erythema can be achieved by sunscreens with very different absorption profiles.** (*a*) These data show the *in vitro* monochromatic protection factors of UVB (octyl methoxycinnamate with $\lambda_{max} = 308$ nm) and UVA (terephthalylidene dicamphor sulfonic acid with $\lambda_{max} = 345$ nm) sunscreen formulations and their common vehicle. Both sunscreen formulations showed an *in vivo* SPF of 4. (*b*) Given that the action spectrum for human erythema shows that UVB is orders of magnitude more potent than UVA (McKinlay and Diffey, 1987), it may seem surprising that a broad spectrum UVA sunscreen can have the same level of protection against erythema as a conventional UVB filter. Weighting (by multiplication) of the sunscreens' absorption spectra with the action spectrum for erythema and the emission spectrum of solar UVR, however, shows that both preparations give maximal protection in the UVB (at about 305 nm) region. As expected, the UVA sunscreen also gives protection in the UVA part of the spectrum.

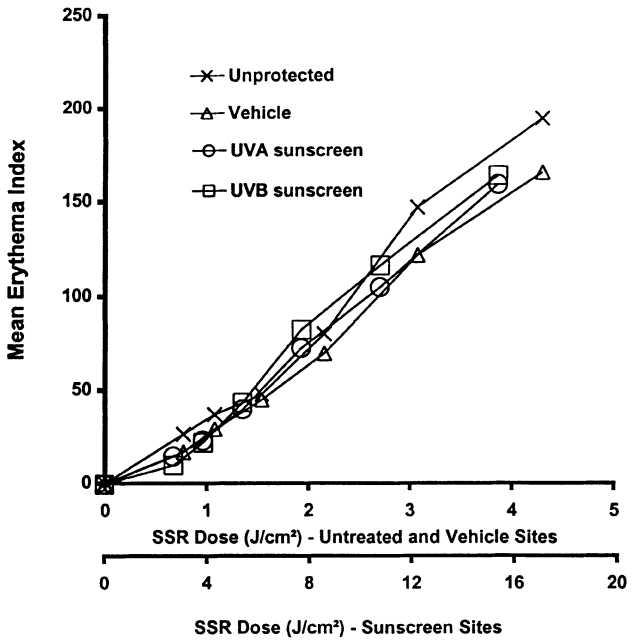


**Figure 3. Erythema dose–response curves for all treatments are the same when an SPF of 4 is assumed.** SPF is normally assessed by comparing threshold doses (MED) with and without sunscreen. These data show 4-fold protection by sunscreens over a range of SSR doses. Note that the reflectance device detects erythema below the visually assessed MED (8/9 with MED of 2.0 J per $cm^2$). SDs not shown for visual clarity.

with thionine. The sections were analyzed using the Discovery automated image analysis system (Becton Dickinson, Leiden, The Netherlands). In each volunteer, the specific background mean optical density (MOD) per nucleus (i.e., TT and (6–4)PP levels from a sample of biopsy obtained from non-SSR exposed and nontopically treated skin) was subtracted from the MOD of the SSR exposed sites.

**Statistical analysis** Statistical analysis was done by two-way analysis of variance.

## RESULTS

**SPF assessment and sunscreen protection from erythema** The vehicle, with a mean SPF of $1.08 \pm 0.36$ (SD) ($n = 9$), had no effect on MED or erythema response determined by the reflectance device, as shown in **Fig 3**. The UVA and UVB sunscreens were shown to have SPFs in the region of 4 ($4.3 \pm 0.8$ (SD) and $3.8 \pm 1.0$ (SD), respectively). These results are close to the SPFs that would be calculated from the monochromatic protection factors, as shown in **Fig 2**(*a*). **Figure 2**(*b*) shows the relative erythemally effective SSR transmitted through each sunscreen normalized at 305 nm. These data show that the UVA sunscreen also gives excellent UVB protection after the appropriate weighting functions for solar UVR and the erythema action spectra have been applied. In addition, the UVA sunscreen gives protection in the UVA region. **Figure 3** shows the erythema dose-response data with all preparations. It is clear that both sunscreens give 4-fold protection against erythema over the SSR dose range used.

**Photoprotection against DNA damage** **Figures 4**(*a*), (*b*) show the processed data using TT and (6–4)PP, respectively. Analysis of variance showed no difference between any of the treatment sites for either TT or (6–4)PP ($p > 0.05$ but mostly in the region of 0.2–0.9). Thus, these results show that (i) vehicle has no effect on DNA photodamage, (ii) 4 MEDs with either sunscreen results in damage comparable to 1 MED without sunscreen (vehicle or no topical application), and (iii) there is no difference in photoprotection between the sunscreens.

## DISCUSSION

We have previously shown that exposure to SSR (without a 1 mm UG 11 filter, which cuts off visible radiation) resulted in dose-dependent increases of TT and (6–4)PP (Young *et al*, 1996). The TT dose–response curve suggested a plateau from about 2–3 MEDs whereas that of (6–4)PP was linear to 4 MEDs. The data in this study show that 4 MEDs of SSR with either sunscreen of SPF = 4 resulted in DNA photodamage (CPD and (6–4)PP) equivalent to 1 MED without sunscreen. We can exclude any modifying effects of CPD repair, which shows large interperson variation (Bykov *et al*, 1999), as biopsies were taken immediately after SSR exposure and therefore before repair is evident (Young *et al*, 1996; Bykov *et al*, 1999). (6–4)PP repair is very much faster than CPD repair and also shows large interperson variation (Young *et al*, 1996; Bykov *et al*, 1999), and some repair may have occurred during the approximately 12 min it took to deliver the 4 MEDs. This may in part explain the larger SDs seen for this photolesion. The sunscreens were designed to have the same SPF, which was verified in our laboratory with our SSR source. The vehicle had no significant effect on the level of erythema or DNA damage. These data strongly suggest that the DNA protection factor is at least as good as the SPF. A similar conclusion can be reached from the data of Freeman *et al* (1988) from a human study that used SSR but did not

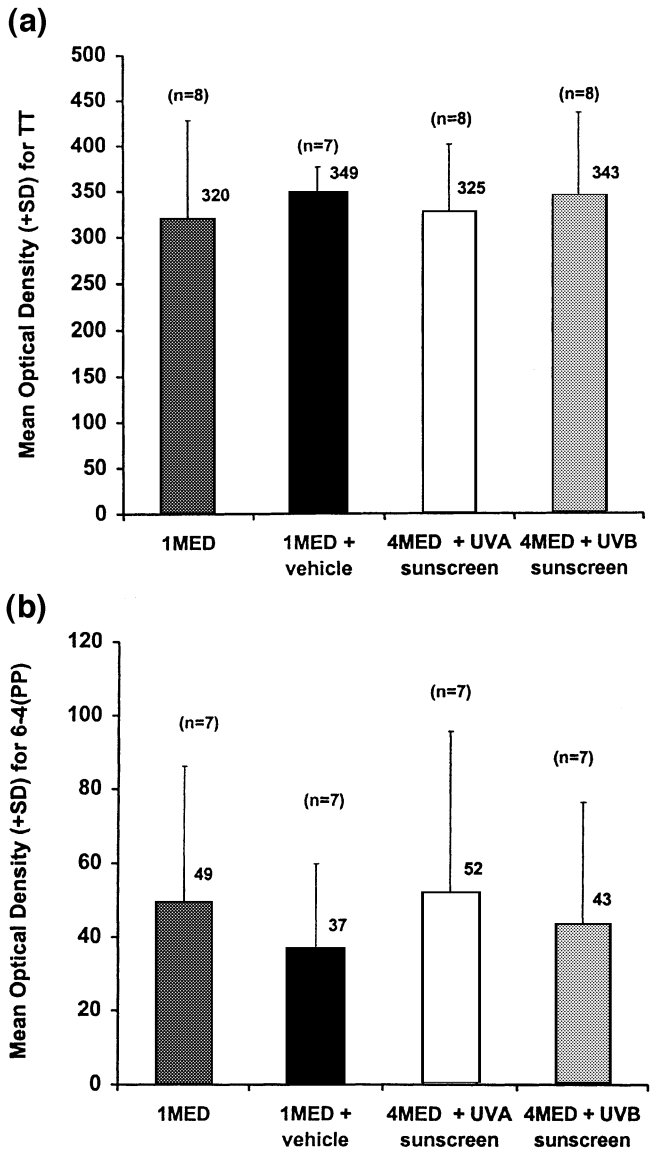


**Figure 4. Exposure of skin to 4 MEDs of SSR plus either sunscreen of SPF = 4 results in the same level of DNA damage as 1 MED ± vehicle.** (*a*) TT. (*b*) (6–4)PP. These results indicate that the DNA protection factor is equivalent to the SPF and provide indirect evidence that DNA is a chromophore for erythema. The larger SD seen with the (6–4)PP data may be the result of the initiation of the repair process, which is rapid and shows large interperson variation (Bykov *et al*, 1999; Young *et al*, 1996). The (6–4)PP antibody also tends to have higher background values, however, than that for CPD in which the signal for a given dose is much larger. Note that the SSR exposed sites have had the 0 MED site background values subtracted on a person by person basis.

confirm labeled SPF with their source. Ideally, good human dose-response data are required to confirm a specific DNA protection factor, but such data are difficult to obtain because of restrictions on the number of biopsies that can be taken from one person. TTs seem to be unimportant in p53 mutation compared with cytosine-containing dimers (Brash *et al*, 1996), but it is reasonable to assume that a sunscreen-related reduction of TTs is also associated with a comparable reduction of cyotsine dimers and cytosine-thymine dimers (Bykov *et al*, 1999). Thus, sunscreens might be expected to afford protection against p53 mutation. This has been demonstrated in mouse skin (Ananthaswamy *et al*, 1997), but protection factors were not determined. In our study, there was no difference between the photoprotective properties of the UVB and the broad spectrum UVA sunscreen formulations, although a similar study in mouse epidermis with the same two formulations showed that the broad spectrum formulation resulted in better protection against CPDs than the UVB product (Ley and Fourtanier, 1997). We do not know the reason for this discrepancy. Our data support a role for human erythema as a good clinical and spectral surrogate for dipyrimidine DNA photolesions. This would be expected if the action spectra in humans for such lesions in epidermis and erythema are the same as described by Young *et al* (1998a). Animal (*Monodelphis domestica*) studies have also suggested that CPD formation is important in erythema (Ley, 1985). More recently, the work of Berg *et al* (1998) suggests that sensitivity to erythema/edema in mice is associated with the inability to carry out transcription-coupled repair of dipyrimidine lesions. It should be remembered that the MED, the endpoint in SPF evaluation, is a threshold on a dose–response curve. Protection equivalent to the SPF across a wide range of UVR doses would be expected if sunscreens act as stable optical filters. This is shown in **Fig 3**. Thus, assuming that erythema is a surrogate for DNA photodamage, sunscreen protection equivalent to SPF would also be expected against a wide range of UVR doses. This remains to be tested, however.

Mouse studies *in vivo* have provided evidence that CPD is a causal factor in UVR-induced immunosuppression (Kripke *et al*, 1992). There is also evidence that the formation of CPD in human cells *in vitro* is associated with the release of tumor necrosis factor α and interleukin-10 that are thought to play an important role in UVR-induced immunosuppression (Kibitel *et al*, 1998; Nishigori *et al*, 1996b). Thus, the data from our study also suggest that sunscreens may afford protection against immunosuppression mediated via DNA photodamage.

In conclusion, our study provides evidence that protection against erythema, whether by a UVB sunscreen or a broad spectrum sunscreen with maximal absorption in the UVA region, is indicative of comparable levels of protection against DNA photolesions at dipyrimidine sites in human epidermis *in situ*. One might speculate that protection from dipyrimidine lesions would also result in comparable protection against mutation. Protection by a broad spectrum UVA sunscreen may offer additional protection from oxidative damage to DNA caused by UVA (Kvam and Tyrrell, 1997) and, more speculatively, malignant melanoma if UVA is confirmed as being important (Setlow and Woodhead, 1994). Comparable protection from sunburn and DNA photodamage with two sunscreens with quite different absorption spectra provides indirect evidence that the action spectra for TT and erythema are the same, as reported by Young *et al* (1998a). Overall, these data suggest that DNA is a chromophore for erythema, which may be regarded as a useful noninvasive clinical surrogate for DNA photodamage. It is important to stress, however, that lack of erythema does not mean lack of epidermal DNA photodamage, which does occur in keratinocytes and melanocytes at suberythemal exposures (Young *et al*, 1996, 1998a, b). Finally, our data support the proper use of sunscreens as one of the means of reducing nonmelanoma skin cancer risk. Such a reduction has been seen in practice (Green *et al*, 1999) but only for the total number of SCC in the study population over a 4.5 y period. This reduction may be expressed as a tumor (SSC) protection factor of 1.6, which is only a tenth of the labeled SPF. Many factors such as prior UVR exposure history, real SPF under conditions of use, etc., however, are likely to be important in the final level of tumor protection. A better understanding of the relationship between SPF, acute protection from skin cancer surrogates, and "cancer protection factor" is an important research objective if we are to understand and predict the likely impact of sunscreen use in skin cancer prevention.

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