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Ultraviolet radiation-induced photoproducts in human skin DNA as biomarkers of damage and its repair

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We have developed a ^{32}P-postlabelling method for quantifying ultraviolet irradiation (UV)-induced cyclobutane dimers and 6-4 photoproducts in human skin *in situ*. We review the application of the method in studies with human volunteers, demonstrating dose–response relationships over a wide range of administered doses, repair kinetics of UV-damaged DNA among healthy individuals and melanoma patients, and modulation by sunscreens, tan and constitutive pigmentation of damage induction. A notable finding is the wide interindividual variation in DNA damage immediately after irradiation and in its repair. Moreover, the protective effects of sunscreens against erythema and DNA damage also show wide interindividual variation. These results cannot be explained by variation in the experimental methods used. The worst-case scenario is that the differences between individuals are multiplicative, resulting in 1000-fold differences in sensitivity in the population, which would be likely to translate into differences in risk of skin cancer.

Introduction

Exposure to ultraviolet (UV) light has deleterious effects on human skin including sunburn, elastoses, cancer and wrinkling. Although only 1–2% of the intensity of the solar radiation reaching the Earth is UVB (280–320 nm), this is believed to be the main cause of the deleterious effects (Sayre, 1992). Some 60% of the sun's energy reaching the Earth's surface is visible light (400–750 nm) and 25% is infrared radiation, i.e., heat. UVA (320–400 nm), accounting for some 15% of the solar energy reaching the Earth, also has deleterious effects but to a smaller extent than UVB. Thus 500–1000 times higher doses of UVA than of UVB are required to cause skin reddening (erythema) (Augustin *et al.*, 1997) and the evidence relating to skin cancer incriminates only UVB (English *et al.*, 1997; Tomatis *et al.*, 1990). Solar energy, and to some extent the emission spectrum, depends on the solar altitude, relating to the time of the day and latitude. The energy in the UVA and UVB range is over four times higher at solar altitude 70° than at 23° (Sayre, 1992).

UVB causes specific types of DNA damage, known as photoproducts, including cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts between two pyrimidines on the same DNA strand (Bykov & Hemminki, 1995). The quantification of specific photoproducts in human skin *in situ* poses many scientific and practical problems. A number of methods are available to measure UV-induced DNA damage, including assays for DNA strand-breaks (Gao *et al.*, 1994; Kasren *et al.*, 1995), unscheduled DNA synthesis (UDS) (Mu & Sancar, 1997), host-cell reactivation (Wei *et al.*, 1993; Runger *et al.*, 1997), immunological detection (Nakagawa *et al.*, 1998) and ^{32}P-postlabelling methods (Liuzzi *et al.*, 1989). Each method has advantages and disadvantages in terms of sensitivity, specificity and practicality. We have developed a ^{32}P-postlabelling method to determine UV-induced DNA damage in human skin *in situ*. One valuable feature of this method is that it can distinguish between the different dipyrimidine combinations of CPDs and 6-4 photoproducts in a single analysis. Another is that a small amount of DNA (less than 3 µg) is needed to analyse the photoproducts,

which is especially useful in molecular epidemiological studies. Using this method, we have studied DNA photodamage and repair in general population samples (Bykov *et al.*, 1998a,b, 1999, 2000) and in people with skin disease (Xu *et al.*, 2000a). Moreover, application of this method to determination of photoproducts in human urine is possible, as described below.

Formation of UV-induced photoproducts is believed to be a crucial event initiating photocarcinogenesis in human skin. However, three evolutionary mechanisms protect against such DNA damage, namely, constitutive pigmentation, DNA repair and tanning. Humans have also invented protective strategies against UV, such as sunscreens, protective glasses, shading, clothes and hats. Thus UV exposures and the induced effects vary widely across the population. Melanoma and squamous cell carcinoma of the skin are the most rapidly increasing types of cancer in Sweden and in many other countries with fair-skinned populations, probably because of changing habits of sunbathing and exposure to UV (English *et al.*, 1997; Center for Epidemiology, 1998). Several lines of evidence suggest that constitutional factors such as skin type, level and type of pigmentation, age, tanning ability and DNA repair capacity partly explain the interindividual differences in sensitivity to photocarcinogenesis (Young *et al.*, 1996; Lock-Andersen *et al.*, 1997; Rosso *et al.*, 1998; Bykov *et al.*, 2000). An extreme example of host susceptibility is xeroderma pigmentosum, a pleiotropic disease in which deficient nucleotide excision repair results in extreme sensitivity to UV and a 1000-fold increase in the incidence of skin cancer (Kraemer *et al.*, 1994; Kraemer, 1997). Even in the general population, photoproduct induction and repair vary substantially among individuals (Freeman, 1988; Bykov *et al.*, 1998b, 1999). Elucidation of the mechanisms underlying interindividual differences in photodamage and its repair will be a key to understanding photocarcinogenesis.

UV-induced DNA damage provides a valuable example in terms of the validation and utilization of biomarkers, for several reasons: (1) the doses can be well controlled and it is ethically acceptable to expose normal humans to doses of about 200–400 J/m^2 that inflict a minimal erythemal response (MED) in a fair-skinned population; (2) UV radiation induces specific types of DNA damage leading to specific tandem CC to TT mutations, also found in defined genes from skin tumours (Harris, 1996); (3) UV radiation is an established cause of human cancer; (4) target tissue is available for experimental studies; (5) the human target tissue is amenable to chemopreventive trials; and (6) an individual DNA repair test can be based on the measurement of removal rates of UV-induced DNA damage. In this chapter we review examples of many of these applications.

Methods

^{32}P-Postlabelling of skin samples

Most of the study subjects were healthy volunteers. However, in two studies cutaneous malignant melanoma patients and healthy controls were used. No significant differences in DNA damage and repair between the two groups were found, thus justifying the pooling of the study populations (Xu *et al.*, 2000a, b). The UV sources used and the doses administered have been described in the cited papers. When solar-simulating radiation was used, the spectral curves mimicked closely the spectrum of solar radiation at the Earth's surface (summer, noontime, Helsinki latitude). Biopsies were taken from buttock skin (usually 4 mm diameter) and were immediately put into ice, frozen and stored at –20 °C to await DNA isolation. DNA extraction from epidermis was performed using a chloroform–isoamyl alcohol method after separation of epidermis from dermis with a blunt scalpel.

The ^{32}P-postlabelling method is based on enzymatic digestion of DNA to nucleoside-3′-phosphates and 5′-labelling of adducts with ^{32}P of high specific activity from [γ-^{32}P]ATP using T4 polynucleotide kinase (Bykov & Hemminki, 1995). One distinct difference in the method for analysing photoproducts compared with other adduct measurements using ^{32}P-postlabelling is that the photoproducts were assayed as trinucleotides with an unmodified nucleotide on the 5′-side. The nucleotide at the 5′-side of each photoproduct can be any one of the four types of nucleotide. The labelled products were detected with a Beckman ^{32}P radioisotope detector and identified by coelution with external standards (Bykov *et al.*, 1999). The level of photoproducts was expressed per 10^6 nucleotides.

In what follows, T=C and T=T designate CPDs, and T–C and T–T designate 6-4 photoproducts.

^{32}P-Postlabelling of urine samples

Approach: Our search for photoproducts is based on the assumption that cyclobutane thymidine dimers (T=T) released by DNA repair are not degraded further and are excreted in urine as a dimer. This was tested by incubating cyclobutane thymidine dimer with S_9 mix enzymes (homogenate from rat liver) at 37°C. After 24 hours, the amounts of unmodified cyclobutane thymidine dimers in the reaction mixture were unchanged, suggesting that the hypothesis was correct, but validation *in vivo* is required.

The cyclobutane thymidine dimer is not a good substrate for T4 polynucleotide kinase and, consequently, cannot be labelled directly. As the parent dinucleotide TpT is easily labelled, we chose to convert the T=T dimer to TpT by UVC irradiation at 254 nm, using a Stratalinker UV Crosslinker 2400 with lamps providing almost monochromatic 254 nm light. This lamp was used both to prepare the reference T=T dimer, and to convert it back to the parent compound TpT. The reversion of T=T to TpT was not quantitative and was quantified in each experiment. Typical yields were 50%.

T=T purification: Urine samples were filtered through 0.22-µm filters and 1 µl was injected onto an HPLC system with UV detection. Preliminary analysis showed that the retention time for the T=T dimer was about 16 minutes, so the fraction eluting between 15 and 17 minutes was collected, freeze-dried and redissolved in 40 µl distilled water. The mixture was then subjected to UVC irradiation (10 kJ/m^2) for conversion of the T=T dimer to the parent dinucleotide (TpT). TpT was labelled on the 5′-side using a protocol described previously (Bykov *et al.*, 1995). The labelled samples were then analysed using an HPLC system with radioisotope detection.

HPLC analysis of TpT: UV-HPLC analyses were performed on a Beckman instrument (model 126 pump) operated with System Gold and coupled to a model 168 diode-array detector (Beckman Instruments, San Ramon, CA, USA). The urine samples were chromatographed on a 5 mm, 4.6 × 250-mm reversed-phase C18 Luna column from Phenomenex (Genetec, Kungsbacka, Sweden). A precolumn filter was positioned before the column. The column was eluted isocratically for 5 minutes with 50 mM ammonium formate buffer (pH 4.6) and then with a gradient from 0 to 30% methanol over 45 minutes at a flow rate of 0.7 ml/min. The labelled samples were analysed with the same Beckman instrument as in the human skin work. However, a different gradient was used: isocratic elution for 3 minutes with buffer (500 mM ammonium formate, 20 mM orthophosphoric acid, pH 4.6), and then a gradient from 0 to 20% methanol over 30 minutes. The identification was based on coelution with standard labelled thymidine dimer.

Results and discussion

Analysis of UV photoproducts required a modification of the postlabelling technique because it was found that cross-linked dinucleotides labelled very poorly (Bykov *et al.*, 1995). In this modification, a normal nucleotide was left on the 5′-side of the cross-linked dinucleotide, resulting in a number of labelled trinucleotides. This is at present the only way to label cross-linked products. Radioactivity was analysed by HPLC with assignment of radioactive products based on the standards used. This assay has been used to study dose–response relationships in humans. In skin biopsies from UV-irradiated skin, there was a linear relationship between dose from 50 to 400 J/m^2 and adduct levels (Bykov *et al.*, 1998a). The relationship was also linear between 150 and 2000 J/m^2 when a sunscreen was used to protect skin, reducing the level of adducts (Bykov *et al.*, 1998b). The amount of photoproducts induced by an MED dose of solar-simulating UV radiation was about 1000 TT=T dimers per 10^8 normal nucleotides (Table 1). This amount is only a quarter of all T=T dimers because equal amounts of dimer would be expected to be formed at all dithymidine sites (TTT, ATT, CTT and GTT), with approximately equal levels at all TC sites. This is a remarkably high level of DNA damage compared with any other known human carcinogen, as shown in Table 1. For example, lung DNA from smokers contains methylation products and benzo[*a*]pyrene types of adduct at levels of only 100 and 10 adducts per 10^8 normal nucleotides, respectively. This high level of UV damage in human DNA is probably the basis for UV-induced carcinogenesis and for the extreme sensitivity to

Table 1. Levels of UV-induced photoproducts in human skin DNA as compared to those of DNA adducts from other exogenous carcinogens that cause cancer in humans

Adduct	Tissue	Level (per 10^8 bases)	Comment
7-Methyl-G	Lung	100	Smoker
7-Hydroxyethyl-G	Leukocyte	100	Smoker
Benzo[a]pyrene-G	Lung	10	Smoker
Benzidine-G	Urothelium	3	Dye workers
PhIP-G	Colon	3	Roasted meat
Tamoxifen-DNA	Endometrium	0.3	Dose c. 40 mg/d
Cyclobutane T=T	Skin	1000	400 J/m^2 UV radiation

Modified from Bykov *et al.* (1998a).
G = guanine, T =thymine.

solar UV-induced skin cancer in xeroderma pigmentosum.

Induction of photoproducts by UV radiation

Irradiation of previously unexposed skin (from buttock) with a defined dose of UV radiation (Xu *et al.*, 2000b) generated high levels of photoproduct detected immediately after irradiation. CPDs and 6-4 photoproducts were formed with different efficiency in human skin, the 6-4 photoproduct levels being about one eighth of those of CPDs. In addition, the levels of photoproducts measured immediately after irradiation showed wide interindividual differences. The levels of each type of photoproduct were correlated with each other and each type showed interindividualvariation approximately proportionate to the absolute level. Figure 1(*a*) shows a 15-fold difference in formation of TT=C (one of the analysed CPDs) between individuals. Up to 30-fold interindividual differences have also been found in previous studies by our group (Bykov *et al.*, 1998b, 1999). The subjects in the present study were melanoma patients and matched healthy controls, but there was no difference between these groups (Xu *et al.*, 2000a). The level of interindividual variation is so large that DNA damage cannot be considered as a simple marker of dose.

Factors modulating UV-induced photoproduct formation

UV-induced DNA damage in skin includes formation of CPDs (TT=C and TT=T) and 6-4 photoproducts (TT–T and TT–C). This is not a random process. A number of factors influence the formation of photoproducts, such as UV wavelength, DNA sequence context and chromosomal proteins (Black *et al.*, 1997; Pfeifer, 1997). We have studied the effects of host factors (e.g., age, skin type, gender) on the formation of photoproducts (Xu *et al.*, 2000b). In older subjects (≥50 years, mean 62.5±9.1 years), the amount of each of the four types of photoproduct immediately after UV irradiation was higher than that in the younger age group (<50 years, mean 42.3±6.6 years) (Table 2). The difference in the level of TT=C reached statistical significance at $p<0.05$. As to skin type, the CPD levels (TT=C and TT=T) were both notably higher in subjects with skin types I/II than in those with skin types III/IV (TT=C was significantly higher, $p<0.05$). However, no clear effect of skin type was found for 6-4 photoproducts (TT–T and TT–C). There was no significant difference between males and females (Table 2).

Multivariate regression analysis showed that age had a systematic effect on the induction of all photoproducts ($0.07 > p \geq 0.05$, data not shown). One year of increased age appeared to cause an increase

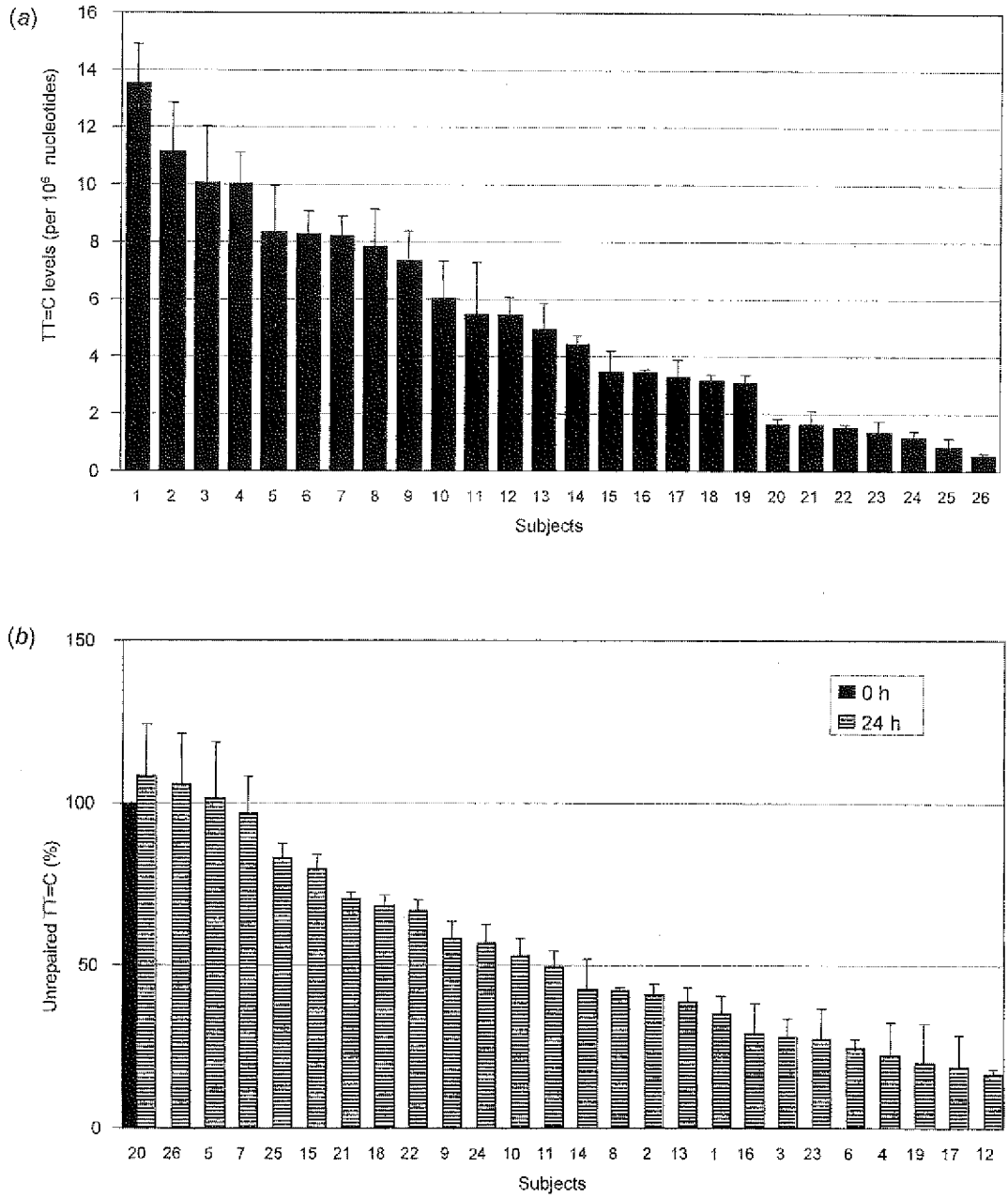

Figure 1. (a) TT=C levels at 0 h after UV irradiation. Bars indicate mean ± SD.
(b) Individual kinetics of TT=C repair in DNA after UV irradiation. The bars show the amount of photoproduct (± SD) remaining in skin DNA 24 h after irradiation. Each point represents the percentage of unrepaired pyrimidine dimers at corresponding time after irradiation.

Table. 2. Photoproduct levels[a] (per 10^6 nucleotides) at 0 hour after UV irradiation

		TT=C	TT=T	TT–T	TT–C
Age (years)	<50	4.22±2.94(15) [b]	5.94±3.58 (16)	0.49±0.33 (16)	0.63±0.46 (16)
	≥50	6.56±3.74* (13)	7.08±3.97 (13)	0.56±0.38 (13)	0.66±0.42 (13)
Skin type	I and II	6.11±3.13* (15)	7.26±4.02 (16)	0.51±0.32 (16)	0.60±0.47 (16)
	III and IV	4.38±3.76 (13)	5.46±3.24 (13)	0.54±0.40 (13)	0.69±0.40 (13)
Gender	Female	5.01±3.50 (7)	6.43±3.68 (7)	0.58±0.28 (7)	0.51±0.36 (7)
	Male	5.40±3.56 (21)	6.46±3.84 (22)	0.51±0.37 (22)	0.68±0.45 (22)

* Student's *t* test, $p<0.05$.
[a] Expressed as mean ± SD
[b] Number of subjects

of about 0.11 TT=C, 0.07 TT=T, 0.01 TT–T and 0.004 TT–C per 10^6 nucleotides. Skin types III and IV, analysed combined, protected against the induction of CPDs (TT=T and TT=C) and of the 6-4 photoproduct TT–T in skin compared with skin types I/II, analysed combined, but the effect was not statistically significant. Thus age was the main host factor influencing the induction of photoproducts in the present study. A plausible explanation may be the changes in skin structure with ageing. In normal skin, ageing alone causes marked changes in morphology, histology and physiology (Gilchrest, 1991). Among these age-associated changes, a decrease of some 10–20% in the number of enzymatically active melanocytes per unit surface area of the skin each decade can result in the reduction of the body's protective barrier against UV radiation (Gilchrest *et al.*, 1979). Thus, considering photoproducts as biomarkers for the risk of skin cancer, the progressively greater UV-induced DNA damage with advancing age may be associated with the steep age effect in incidence of squamous cell carcinoma of the skin. In Sweden, the incidence of this cancer is highest in the age groups over 85 years, exceeding that in the 60–64-year age group by more than 10 times (Center for Epidemiology, 1998).

Repair of photoproducts

UV-induced DNA damage and many bulky DNA lesions are repaired by means of the nucleotide excision repair (NER) enzyme system, which involves about 30 different gene products (Lehmann, 1995). NER involves two sub-pathways, global genomic repair and transcription-coupled repair. Repair of photoproducts by NER also shows heterogeneity: photoproducts are not repaired with the same efficiency within all regions of the genome (Black *et al.*, 1997; Pfeifer, 1997). In the present study, the global genomic repair of photoproducts displayed substantial interindividual differences and sequence-dependence (Xu *et al.*, 2000b). Taking the CPD TT=C as an example, the percentage of repaired photoproducts at 24 h after irradiation varied from zero to about 82% among the study population. Figure 1(b) shows the amount of TT=C remaining in skin DNA of 26 individuals 24 h after UV irradiation. The subjects were melanoma patients and matched healthy controls, but there was no difference between these groups (Xu *et al.*, 2000a). It is clear that the rate of repair bears no relationship to the initial level of photoproducts. There was evidence of photoproduct-specific repair, with TT=C showing a faster rate of repair than TT=T: 46% and 80% of TT=C was repaired within 24 h and 48 h after irradiation, respectively, compared with 25% and 54% for TT=T.

We assessed the effects of some host factors that may influence the rate of repair of photoproducts (data not shown). Age (range 32–78 years) had no consistent significant effect on the repair rate of TT=C or TT=T up to 48 h after irradiation (Xu *et al.*, 2000b). No effect of skin type or gender on DNA

repair was found. In contrast, one early study showed an age-related decline in DNA repair capacity in human lymphocytes *in vitro* (Wei *et al.*, 1993). The discrepancy between the results could be due to factors such as methodological differences, repair capacities *in situ* vs *in vitro*, or differences between cell types. However, for repair of UV-induced DNA damage in human skin, only the present results are directly relevant. In all these studies, we measured the rate of removal of photoproducts, rather than direct repair. However, these photoproducts are chemically stable in DNA and require DNA repair for their removal. Cell replication would cause an increase in amounts of DNA and thus dilution of the photoproducts. We cannot rule out some minor effect of DNA replication, despite the short duration of the studies (up to two days).

Effect of sunscreen and tan

We have applied the postlabelling method to assess the effects of sunscreens and tanning on UV-induced DNA damage in human skin *in situ* (Bykov *et al.*, 1998b). Sunscreen was applied by one person, according to the manufacturer's instructions. While the protection against the erythemal response varied fivefold among nine subjects, protection against DNA damage differed by a factor of 10 and was independent of the erythemal response. On average, sunscreens protected against DNA damage in accordance with the sun-protection factor (SPF), but the degree of protection was highly individual. Figure 2 shows the individual SPF (ISPF) and the protection factor against DNA damage (PF/DNA damage); thus, for individual L, sunscreen provided an ISPF of 20 but the protection against DNA damage was only about 5. For individual C, the reverse was the case. Since the SPF is based on average erythemal response, it is no guarantee against individual DNA damage. It is likely that the main reason for the apparent interindividual difference is simply uneven spreading of the sunscreen. Figure 2 also shows that even

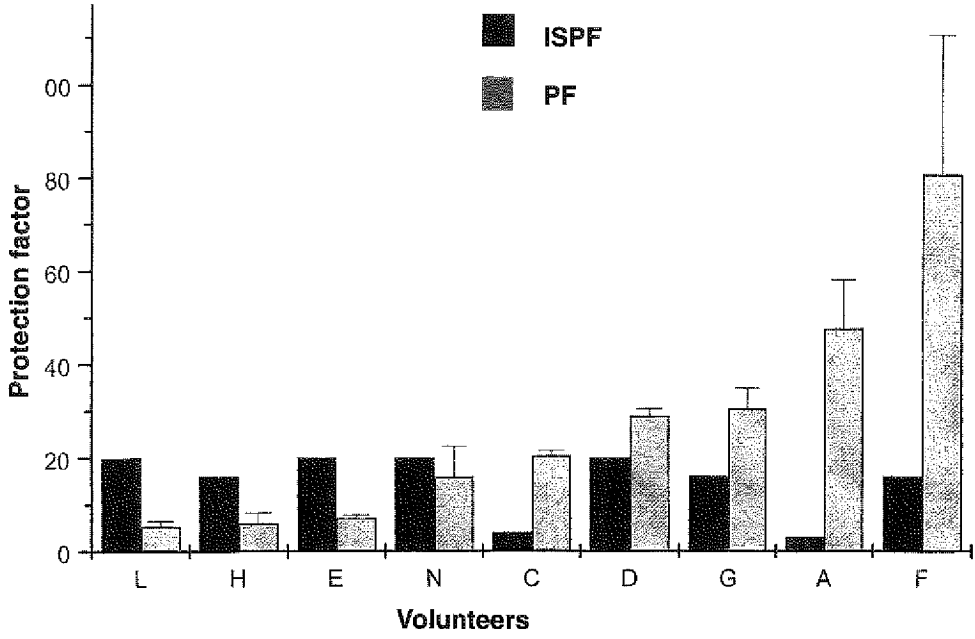

Figure 2. Individual sunscreen protection factor (ISPF), based on erythemal response, and protection factor against DNA damage (PF/DNA damage) measured in nine subjects who received 150 J/m^2 of UVB. Data from Bykov *et al.* (1998b).

The SPF of the sunscreen was 10.

protection against the erythemal response (ISPF) is highly individual.

Tanning provides an endogenous 'sunscreen' with an SPF of 3 to 5 (Gilchrest *et al.*, 1999). These measurements are based on protection against erythema, and fundamental data on how well tanning protects genetic material appear to be lacking. We have investigated the effects of tanning on DNA damage *in situ*, under conditions simulating the use of sunbeds (Hemminki *et al.*, 1999; Bykov *et al.*, 2000). We measured the protective effects of tanning by quantifying the levels of UV-induced photoproducts in skin of eight healthy, fair-skinned Caucasians. Each subject was irradiated on a 2 x 4-cm area of skin on the lower back with UVB radiation at a dose of 0.3 J/cm^2 and on a second 2 x 4-cm area at a dose of 0.6 J/cm^2. A punch biopsy was performed immediately after irradiation. An additional biopsy was performed on unirradiated skin and served as a background control. Tanning was induced by 10–13 sessions of UVA irradiation for three weeks. Tanning was observed by a clear change in skin colour towards brown and was measured with a reflectometer adjusted to record melanin pigmentation. In the course of UVA treatment, the instrumental readings indicated an increase in pigmentation of 38.8 ± 16.7 reflectometer units (mean ± SD, $n = 8$). After the last UVA dose, the challenge with UVB was repeated, and three biopsy specimens were taken, as described above, except that the control biopsy was from tanned skin. The samples were coded for blind analysis. Photoprotection was defined as the difference in photoproduct levels before and after the UVA treatment.

In subjects who received 0.6 J/cm^2 of UVB, the levels of CPDs were slightly lower than those in untanned skin (Figure 3). The average tanning protection factor was only 1.19 ± 0.17 (mean ± SD). Since tanning acts like a low-level sunscreen to suppress the erythemal response without the unpredictable and limited protection against DNA damage afforded by a chemical sunscreen (Bykov *et al.*, 1998b), people who have acquired a tan may prolong sun exposure, resulting in DNA damage and increased risk of skin cancer. Tanning may

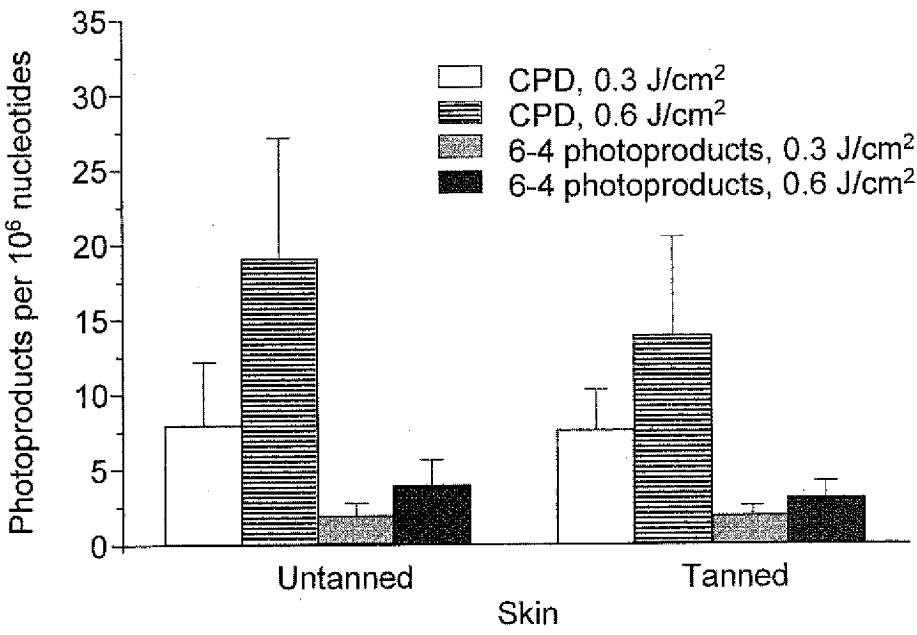

Figure 3. Induction of photoproducts in untanned and tanned skin

Levels of UV-induced DNA damage, cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts are expressed per 10^6 nucleotides (mean ± SD, $n = 8$ subjects). From Hemminki *et al.* (1999).

provide a false sense of security, leading to inadvertently lengthened recreational sun exposure like that of the high-SPF sunscreen users studied by Autier *et al.* (1999). Natural pigmentation, even in the fair-skinned Scandinavians, provides better protection against UV-induced DNA damage than UVA-induced tanning (Bykov *et al.*, 2000). Sun-induced tanning may also provide better protection against DNA damage than that induced by UVA. However, solar-simulated tan in skin types II and III afforded a protection factor of only 2 against erythemal response (Sheehan *et al.*, 1998).

Urinary photoproducts

The amount of CPDs that can theoretically be expected in urine was estimated using the following assumptions: (1) a dose of 400 J/m^2 of solar-simulating radiation (1–2 MED, about 15–30 min sun in Stockholm area in summer) induces the formation of 6 TT=T per10^6 nucleotides in skin DNA, giving a total amount of dimer (AT=T, GT=T, CT=T and TT=T) of 24 T=T per 10^6 nucleotides; (2) the amount of DNA extracted from a skin biopsy is 10 µg, correcting for yield; thus 1 m^2 of skin will contain 800 mg or 60 nmol T=T; (3) 25% of T=T is removed from skin DNA in the first 24 hours after UV irradiation (Xu *et al.*, 2000b); (4) all T=T

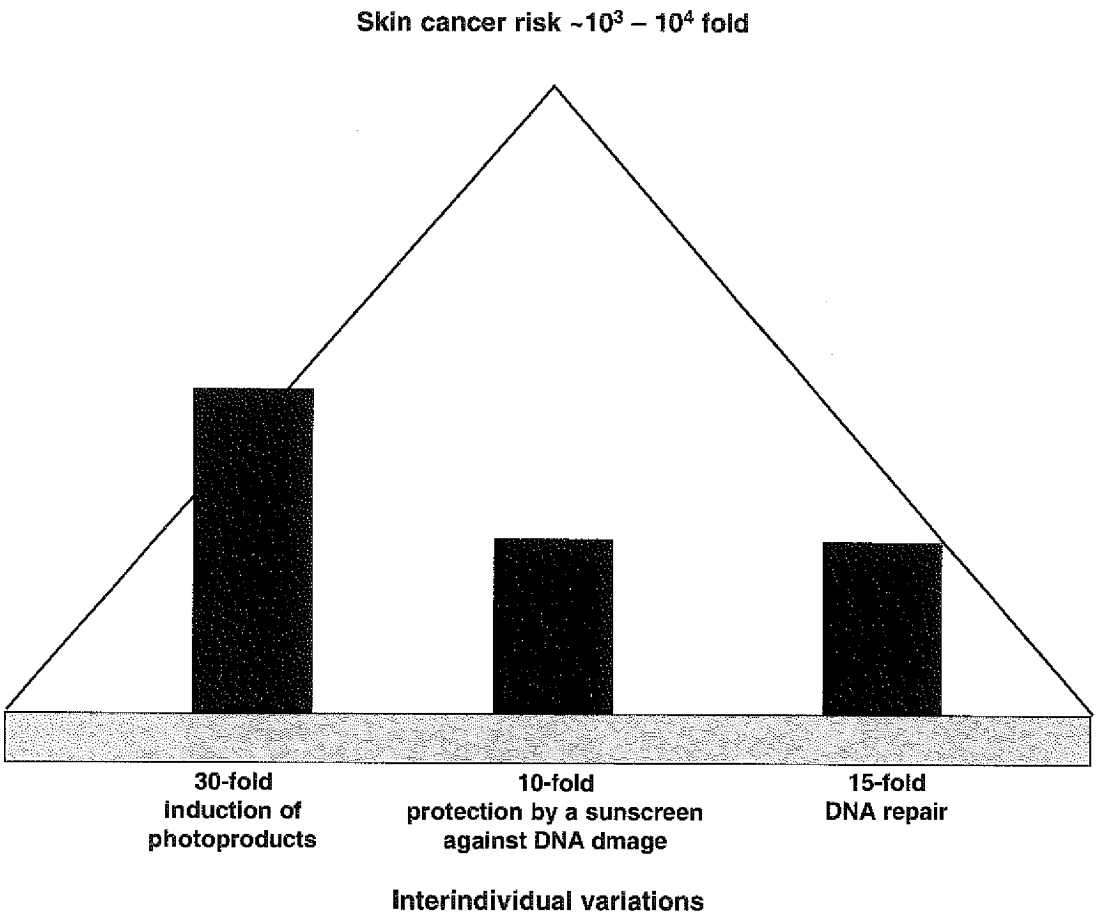

Figure 4. A scheme of the interindividual variations observed in the studies cited.

removed from skin DNA ends up in urine (1 litre per 24 h). This calculation indicated that the level of T=T to be expected in urine is 15 fmol/μl.

In chromatographic analyses of urine samples containing CPDs, the ^{32}P-labelled TpT showed a retention time of about 27 minutes. The first analyses of human urine collected after sun exposure showed T=T levels of 2 to 20 fmol per μl of urine. These amounts are consistent with the theoretical levels calculated above. If validated, this ^{32}P-postlabelling quantification of thymidine dimers in urine would provide a non-invasive method to assess the levels of photoproducts after whole-body exposure to UV light, that would offer an easy alternative to skin biopsies.

Conclusions

A surprising finding in these studies has been the large interindividual variation in the immediate DNA damage after exposure to UV and in its repair. Moreover, the protective effects of sunscreens against erythema and DNA damage also show wide interindividual variation. These results cannot be explained by variations in the experimental methods used. While the interindividual variation in the levels of immediate DNA damage can be 30-fold, our coefficient of variation among repeated analyses of the same samples was only some 30% (Bykov *et al.*, 2000). An interesting issue is the stability of individual response to UV radiation. So far we have limited data on this: there was a high correlation in the induced photoproduct levels when the same individuals were tested three weeks apart (unpublished data). Figure 4 illustrates the ranges of interindividual variations found in these relatively small studies. The worst-case scenario would be that the various differences are multiplicative, resulting in 1000-fold differences in sensitivity between individuals. These would be likely to translate into differences in risk of skin cancer.

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