Ultraviolet B-induced DNA Damage in Human Skin and Its Modulation by a Sunscreen

Vladimir J. Bykov, Jan A. Marcusson, and Kari Hemminki

Department of Biosciences at Novum, Karolinska Institute, Novum, 141 57 Huddinge [V. J. B., K. H. ], and Department of Dermatology, Huddinge Hospital, 141 86 Huddinge [J. A. M.], Sweden

Abstract

The UVB component of solar radiation is a risk factor for skin cancer, the most common cancer in the Western world. Yet little is known about the induction of DNA damage in human skin by UVB and its modulation by sunscreens. Here, we apply a novel postlabeling high-performance liquid chromatography technique to quantify UVB-induced photoproducts in skin biopsies with and without sunscreen. The results showed ~30-fold interindividual variations in levels of DNA damage in unprotected skin of the 14 subjects, probably relating to skin cancer susceptibility. On average, sunscreen guards against DNA damage as expected by the erythema response, but some individuals are poorly protected.

Introduction

Sunscreens are beneficial to skin because they reduce UV-induced DNA damage (1, 2), lower mutation frequency (3), and inhibit phototaging (4). Sunscreens may also efficiently reduce the frequency of actinic keratosis, a precancerous lesion (5, 6). Thus, sunscreens appear to be useful in preventing skin cancer (7). The potency of a sunscreen is determined by a SPF, defined as the ratio of the energy required to produce a MED (skin reddening or minimal sunburn) through the sunscreen compared with the energy required to produce the same reaction in the absence of the sunscreen (8). The efficacy of a sunscreen in preventing erythema may encourage longer sun exposure, which may compromise the beneficial effects (9). Although there is no doubt that sunscreens are able to reduce many effects of solar radiation, it is unclear how well the protection against erythema guards against DNA damage.

We have developed a method enabling, for the first time, direct identification and quantitation of UV-induced photoproducts in human skin biopsies (10–12). The method measures both cyclobutane pyrimidine dimers and 6-4 photoproducts, the major causes of the lethal (13), mutagenic (14–17), transforming (18), and carcinogenic (19, 20) properties of UV irradiation. We apply this technique here to quantify individual skin response to experimental UV irradiation and modulation of the response by sunscreen and prolonged exposure.

Subjects and Methods

Volunteers. Fourteen healthy Caucasian volunteers (7 females and 7 males) with skin types I and II (21) were recruited from medical students and laboratory staff at Huddinge Hospital. The study plan was approved by the local ethical committee, and the volunteers gave their informed consent to participate. The ages of the volunteers varied from 18 to 56 years (median age, 26 years old).

UV Radiation. Waldmann F 85/100 W-UV21 tubes, mounted in a Waldmann UV 3003 K screen (Waldman, Germany) with automatic computerized light dose measurements, were used for UVB irradiations. This is a commonly used clinical lamp covering a UVB area of 280–315 nm, emitting ~5% of the relative spectral irradiation output in wavelengths of <290 nm and hardly any in wavelengths of <280 nm.

For experiments, we chose a commercially available sunscreen, pH5-Eucerin (Beiersdorf AG, Germany), with a stated SPF of 10 and a stated ability to filter out both UVB and UVA. The sunscreen was applied as internationally agreed, at a thickness of 2 μl/cm² (22) on a lumbar part of back. For a testing of MED with and without a sunscreen protection, the following single doses of UVB were used: 50, 100, 200, and 400 J/m² on unprotected and protected sites and, additionally, 800 and 2000 J/m² on sunscreen-protected areas. MED was assessed after 24 h according to the following grading: 0, no erythema at all; (+), mild erythema with partially defined edges (two edges or less); (+), uniform erythema with sharp borders and 3 or more clearly visible edges that were considered MED; ++, clear red color with slight induration; ++++, heavily red color with palpable edema. The test areas were squares measuring 2 cm × 3 cm. The areas were irradiated at 150, 150, 370, 920, and 2000 J/m² and biopsied just below the waistline in the back. The latter four sites were covered with a sunscreen before the irradiation. Biopsies from one uniradiated area were included as a background. Five volunteers were biopsied 5 times, excluding the 2000 J/m² irradiated site, and nine volunteers were biopsied 6 times. After an intradermal injection of 5 mg/ml xylocaine-adrenaline (Astra AB, Södertälje, Sweden), 4-mm punch biopsies were collected. The time between irradiation and a biopsy excision was <15 min. The specimens were put in a plastic tube and rapidly frozen in carbon dioxide ice. The material was stored in a freezer at −20°C until analysis. The sample codes were blinded until completion of the analyses.

DNA Extraction and Photoproduct Analysis. DNA was isolated as described (12). From each 4-mm punch biopsy, we could isolate 4–8 μg of DNA. For each 3P-postlabeling assay, 3 μg of DNA were digested with enzymes that produced photoproduct-containing trinucleotides and nucleosides (10, 11, 23).

Photoproduct standards were synthesized from nonmodified trinucleotides (23). The standards of measuring photoproducts were mixed with hydroxylated nonmodified DNA and labeled together with the human samples. The quantification took into account the amount of a standard added and a peak area obtained from human samples. The labeling efficiency at the conditions used varied: ~100% for TT=T and 80% for TT=C (cyclobutane pyrimidine dimers) and 70% for TT=T and 30% for TT—C (6-4’-[pyrimidine-2’-one] pyrimidine photoproducts) (Refs. 10, 11, and 23). The reproducibility of assay was calculated as percentage of a difference between two measurements divided by their mean. The average value was 56% for the detected photoproducts.

All statistical calculations were performed with Origin Microcal software.

Results

The 14 volunteers had skin types I and II and showed a MED of 50–100 J/m² in 24 h. Two cyclobutane dimers and two 6-4 photoproducts were measured in their skin biopsies following UVB irradiation using the postlabeling-high-performance liquid chromatography method. The photoproducts were measured as trinucleotides, in which...
the 5' nucleotide was unmodified and the two 3' nucleotides were linked by the cyclobutane (marked as =) or 6-4 bond (marked as —¿). The measured trinucleotides were TT=C, TT=T, TT—C, and TT—T. Each represents one-fourth of the possible photoproduct in DNA; e.g., TT=T is one-fourth of the possible photoproducts TT=T, CT=T, AT=T, and GT=T. However, we only give the results for the particular trinucleotide measured.

The mean skin responses and SEs among the 14 volunteers to 150 J/m² are shown in Fig. 1. Cyclobutane dimers (TT=C and TT=T) were formed in unprotected skin at ~2.5 photoproducts/10⁶ nucleotides and were ~5 times more abundant than 6-4 photoproducts (TT—C and TT—T). The sunscreen of SPF 10 reduced the level of the adducts to ~1/20, somewhat more than expected by the SPF 10 factor. The sunscreen reduced the levels of the different photoproducts to an equal extent. The reduction of each photoproduct by the sunscreen was highly significant statistically (P < 0.001, paired t test).

The individual skin responses to 150 J/m² are shown in Fig. 2 with and without the sunscreen of SPF 10. Only the levels of TT=C in DNA are given in Fig. 2 because there was a good correlation among the four photoproducts within individuals (r = 0.7–0.99). There was an astonishingly large difference in the individual response to UVB, spanning a range of >30 fold for the cyclobutane adducts, which were present in all of the samples (for some individuals, no 6-4 photoproducts were detectable). Remarkably, there was no correlation between the photoproduct levels between the unprotected and protected skin sites (r = —¿0.07). For example, volunteers A, C, H, L, and O were very effectively protected, whereas volunteers G, M, and N received little benefit from the sunscreen. We observed no effect of age or sex on photoproduct levels.

The volunteers received increasing doses of UVB on the sunscreen protected skin, up to 2000 J/m² (Fig. 3). The induction of photoproducts increased approximately linearly with increasing dose, but at only ~1/10–1/20 of the response expected in unprotected skin (compare to the 150 J/m² dose; Fig. 3, left). The solid line shows the expected photoproduct levels, assuming that DNA damage were reduced to 1/10 by sunscreen. Thus, the sunscreen performed as expected at the group level, but as seen in the large error bars, the large interindividual variability persists at all dose levels. The erythemal response in the protected area was checked in nine volunteers. The sunscreen had been effective in all of them (observed SPF varied between 3 and 20).

The volunteers received increasing doses of UVB on the sunscreen protected skin, up to 2000 J/m² (Fig. 3). The induction of photoproducts increased approximately linearly with increasing dose, but at only ~1/10–1/20 of the response expected in unprotected skin (compare to the 150 J/m² dose; Fig. 3, left). The solid line shows the expected photoproduct levels, assuming that DNA damage were reduced to 1/10 by sunscreen. Thus, the sunscreen performed as expected at the group level, but as seen in the large error bars, the large interindividual variability persists at all dose levels. The erythemal response in the protected area was checked in nine volunteers. The sunscreen had been effective in all of them (observed SPF varied between 3 and 20).
Discussion

Considering that skin cancer is the most common cancer type in Western countries and that solar UVB is an important risk factor, the lack of human data on UVB-induced DNA damage and its modulation by sunscreens is surprising. Two relevant previous papers evaluated a total of 11 individuals with indirect techniques, noting a clear effect of the sunscreen (1, 2). Here, we apply, for the first time, a direct technique on 14 individuals, analyzed in blinded samples, and find two unexpected results. For one, there is a remarkable 30-fold difference in the levels of photoproducts in unprotected skin among the individuals after a uniform dose of 150 J/m². For the other, the protection by the sunscreen appeared to be very individual and entirely independent of the magnitude of the DNA damage in unprotected skin or erythemal response. All of the subjects were fair-skinned, of skin types I and II, with recorded MEDs of 50–100 J/m². The only previous indication of individual sensitivity is our pilot study on eight volunteers, who also exhibited a large interindividual variation in response (12). The interindividual variation in this study was far larger than any methodological variation, which was ~56% in repeated analyses of the same samples. Although the sunscreen of SPF 10 provided a uniform reduction in the level of photoproducts by 90–95%, as expected based on the erythema protection, over a dose range of 150–2000 J/m², the individual responses varied from 99 to 65%. The results reported here are based on one commercial sunscreen only but we also have tested this sunscreen with SPF 25 and two different sunscreens with SPF 10 and 25 from the same company and obtained similar results.

One qualification regarding the results is the UV source used. This was a commonly used clinical lamp covering a UVB area of 280–315 nm, emitting ~5% of the relative spectral radiation output in wavelengths of <290 nm, with hardly any being <280 nm. Although the emission from this source at wavelengths of <300 nm was relatively higher than that from solar-simulated sources, the production and distribution of photoproducts in human skin were quite comparable (12). Interestingly, the protection from photoproducts by the sunscreen of SPF 10 used here was, on average, close to a factor of 10 over the wide dose-range applied here.

The results have compelling implications for UVB-induced skin carcinogenesis, assuming that DNA damage is the initial trigger. First, the effective DNA doses resulting from sun exposure may be highly individual. If such a sensitivity persists over a lifetime, the DNA damage inflicted by tanning in fair-skinned people may vary by two orders of magnitude, at least. Because DNA damage is independent of erythema, there is no natural avoidance against tanning. The relationship, if any, of such a suggested sensitivity to the appearance of multiple skin cancers in individuals without family histories may provide interesting mechanistic leads. The reasons for the individual response remain unclear but probably relate to the structure of skin. We had no data on how the photoproducts actually distributed in the epidermal cells. The recoveries of DNA from the skin biopsies were rather uniform at 4–8 μg. Removal of photoproducts by nucleotide excision repair could not affect the results because the biopsies were placed on ice immediately after irradiation. Apparently, human skin contains agents that can modulate the effect of UVB on DNA, either by protecting or enhancing. For example, an equal dose of UVB caused more photoproducts in cultured skin than in DNA in solution, as was shown by the present technique recently (11).

A second implication of these results is that the individual variability in the protective effects of the sunscreen against DNA damage raises concerns that protection against erythema by the sunscreen is unrelated to the extent of DNA damage. It should be emphasized that the sunscreen was applied uniformly by one person, and the erythema response recorded in the sunscreen protected area confirmed the efficacy of the screen against erythemal response. Such uniform application of the sunscreen in routine use is probably rare. On the basis of mitigated erythema response, sunscreens may encourage prolonged sun exposure among fair-skinned persons. The consequences may be most unfortunate to those whose DNA is poorly protected by the sunscreen.

References

Ultraviolet B-induced DNA Damage in Human Skin and Its Modulation by a Sunscreen

Vladimir J. Bykov, Jan A. Marcusson and Kari Hemminki

*Cancer Res* 1998;58:2961-2964.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/14/2961

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.