Induction of Cyclobutane Pyrimidine Dimers, Pyrimidine(6–4)pyrimidone Photoproducts, and Dewar Valence Isomers by Natural Sunlight in Normal Human Mononuclear Cells

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Abstract

Immunocytochemistry was used for the direct measurement of cyclobutane pyrimidine dimers, (6-4) photoproducts, and Dewar isomers in normal human mononuclear cells following irradiation by natural sunlight or by a FS20 broad spectrum UVB sunlamp. The induction of each type of photoproduct was detected following 30–60 min sunlight exposure or with FS20 fluences as low as 50–100 J m⁻². With increasing FS20 fluences, there was a dose-dependent increase in the binding of cyclobutane dimer, (6-4) photoproduct, and Dewar isomer-specific monoclonal antibodies. The relative ratio of Dewar isomer to (6-4) photoproduct antibody binding sites was much higher following exposure to natural sunlight than to broad spectrum UVB. With the (6-4) monoclonal antibody, a small increase in binding sites was evident after a 1-h exposure to natural sunlight. This remained relatively constant with further exposure. These results are consistent with the hypothesis that, following irradiation with natural sunlight, the majority of (6-4) photoproducts are converted into Dewar valence isomers.

Introduction

There is overwhelming evidence implicating increased exposure to the UV component of natural sunlight as the major cause of human skin cancer (1). Although the precise mechanisms have not been established, it is accepted that UV-induced DNA damage is an important underlying factor. Analysis of DNA isolated from skin tumors reveals that the majority of mutations occur at dipyrimidine sites, especially at TC and CC sequences. Solar UVR is subdivided into three regions: UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm). Due to the limitations of working with sunlight, most laboratories investigating the biological effects of UV have used artificial sources and, in particular, germicidal UVC lamps emitting predominantly at 254 nm. UVC contains the most potent wavelengths for inducing DNA damage. The major types of damage induced by UVC are CPDs and pyrimidine(6-4)pyrimidone photoproducts. These are formed by the direct absorption of UVC by DNA at dipyrimidine sites and constitute approximately 70–80% and 20–30% of UVC-induced DNA damage, respectively. It has been established that both types of photoproducts contribute significantly to the UVC-induced genotoxicity and mutability of human and rodent cells (2). However, UVC does not reflect the quality of solar UVR to which living cells are generally exposed. Wavelengths lower than 290 nm do not reach the earth’s surface due to absorption by atmospheric gases and attenuation by stratospheric ozone. Consequently, sunlight reaching the earth’s surface contains approximately 0.3% UVB (>290 nm), 4.7% UVA, 40% visible light, and 55% infrared radiation (1). The adverse effects of natural sunlight on living systems are largely attributable to the small amount of UVB that is absorbed by cellular DNA. UVA wavelengths are less efficient at inducing DNA damage. They are not absorbed by native DNA but can produce secondary photo reactions of existing DNA photoproducts or damage DNA via indirect photosensitizing reactions (3, 4).

UVB induces a different spectrum of DNA damage than does UVC since it produces a significant yield of Dewar isomers (5). These are formed by the photoisomerization of (6-4) photoproducts by wavelengths longer than 290 nm (Fig. 1; Refs. 3 and 6). Since the photoisomerization is most efficient around 320 nm (which corresponds to the UV absorption maximum of (6-4) photoproducts), UV sources containing a higher proportion of radiation bordering between UVB and UVA, such as solar UVR, should produce a higher proportion of Dewar isomers. Consequently, it has been suggested that all (6-4) photoproducts will be converted into Dewar isomers upon exposure to sunlight (7). We have, therefore, measured the induction of pyrimidine dimers, (6-4) photoproducts, and Dewar isomers in normal human mononuclear cells following irradiation by natural sunlight. This is the first report for the simultaneous detection of pyrimidine dimers, (6-4) photoproducts, and Dewar isomers in human cells by natural sunlight and is consistent with a biological role for Dewar isomers.

Materials and Methods

Cell Culture. Human mononuclear cells were separated on a Ficoll gradient from freshly donated blood and stored frozen in liquid nitrogen according to standard procedures (8). When required, cells were thawed and incubated overnight at 37°C under 5% CO₂ at 10⁶ cells ml⁻¹ in RPMI 1640 (GIBCO-BRL, Paisley, United Kingdom) containing 10% pooled human AB serum. Prior to UV irradiation, cells were pelleted, washed, and resuspended at 10⁵ cells ml⁻¹ with RPMI 1640 without phenol red or 10% AB serum.

UV Irradiation. For natural sunlight irradiation, 10⁶ cells were irradiated as 1 ml droplets in the center of bacterial grade 5-cm plastic Nunclon Petri dishes (Life Technologies, Ltd., Paisley, United Kingdom) while being held at approximately 4°C on ice. In three independent experiments, cells were irradiated under direct sunlight on three fine days during summer (July 27th and August 3rd and 16th, 1994) outside the MRC Cell Mutation Unit (Brighton, England: 50°N-0.1°W) between noon and 4:00 p.m. Surface temperatures typically ranged between 22–34°C. UVB and UVA fluence rates were estimated using a UVX portable radiometer (UV Products, San Gabriel, CA) with interchangeable sensors to measure incident UVB fluences with a peak response at 310 nm and incident UVA with a peak response at 365 nm. Fluence rates were typically between 2–9 J m⁻² s⁻¹ and 10–24 J m⁻² s⁻¹, respectively, depending upon variation with climatic conditions.

For broad spectrum UVB irradiation, 5 × 10⁶ cells were irradiated with a bank of three Westinghouse FS20 sunlamps as 0.5 ml droplets through the...
bottom of bacterial grade 5-cm plastic Petri dishes that eliminate wavelengths shorter than 290 nm. For comparison with previous results (5, 9), FS20 fluence rates were measured with an International Light IL 1350 radiometer with a SCS280 filter (Able Instruments, Reading, United Kingdom) and were typically 2–3 J m⁻² s⁻¹. FS20 fluorescence rates measured with the UVX radiometer and 310 nm sensor were approximately 2.4 times greater. The spectral output of the SCS280 filter (Able Instruments, Reading, United Kingdom) and were typically 2–3 J m⁻² s⁻¹. FS20 fluorescence rates measured with the UVX radiometer and 310 nm sensor were approximately 2.4 times greater. The spectral output of the FS20 sunlamp is shown in Fig. 2.

**Immunostaining.** Induction of pyrimidine dimers, (6–4) photoproducts, or Dewar isomers was detected using H3 (10), 64M-2 (11), and DEM-1 (3) mouse monoclonal antibodies, respectively. To detect lesions induced by low UV fluences, a biotin-streptavidin immunostain was adopted. Essentially, the protocol was as outlined elsewhere (5, 9) but with minor modifications. Briefly, following UV irradiation, cells were fixed and incubated with the primary lesion-specific antibody as described. Biotinylated F(ab')₂ fragment of rabbit antimouse immunoglobulins (DAKO Ltd., High Wycombe, United Kingdom) was applied at a 1:150 dilution with Tris-buffered saline [20 mM Tris-HCl, 150 mM NaCl (pH 7.4), and 0.2% BSA] for 30 min at 20°C, followed successively by alkaline phosphatase-conjugated streptavidin (DAKO Ltd., High Wycombe, United Kingdom) at a 1:75 dilution for 30 min and fast red substrate [1 mg/ml in 0.1 M Tris-HCl (pH 8.2) containing 0.02% naphthol phosphate and 2% dimethylformamidyl] for up to 20 min. The amount of the insoluble red reaction product produced at the site of the target antigen was quantified by area densitometry using automated image analysis and was defined by an arbitrary gray scale value as described previously (5, 9).

**Results**

The dose-dependent binding of H3, 64M-2, and DEM-1 antibodies to the cellular DNA of normal human mononuclear cells following irradiation with a broad spectrum UVB sunlamp or natural sunlight is presented in Fig. 3. The biotin-streptavidin labeling system was approximately twice as sensitive as the method described previously (10). After irradiation with broad spectrum UVB, there was a dose-dependent increase in the binding of pyrimidine dimer H3 (Fig. 3A), (6–4) photoproduct 64M-2 (Fig. 3B), and Dewar isomer DEM-1 (Fig. 3C) specific monoclonal antibodies with increasing FS20 fluences. In contrast, only the pyrimidine dimer (Fig. 3D) and Dewar isomer (Fig. 3F) antibodies were found to exhibit dose-dependent binding following irradiation with natural sunlight. With the (6–4) monoclonal antibody (Fig. 3E), a small increase in binding sites was evident after 1-h sunlight exposure; this did not increase further but remained relatively constant throughout the duration of irradiation. Each data point represents the mean of three independent experiments and shows the mean ±SE.

**Discussion**

We have shown previously that for normal human fibroblasts, (6–4) photoproducts are the major cytotoxic lesion following irradiation with artificial UVC and UVB lamps (5). However, the results presented in this study are consistent with the hypothesis that, following exposure of mammalian cells to natural sunlight, the majority of (6–4) photoproducts are converted into their Dewar valence isomers (7). This raises the question as to whether the low level of (6–4) photoproducts or the greater yield of Dewar isomers play a more important role in mutation and the induction of skin cancer under physiological conditions and exposure to solar UVR.

Action spectra for the induction of CPDs in naked DNA show that their formation is most efficient around 260 nm (12). In human epidermal skin cells, the most efficient wavelengths for the formation of CPDs are around 300 nm (13). This is due to the greater attenuation of shorter UV wavelengths by the outer stratum corneum and epidermal skin layers. The formation of (6–4) photoproducts in naked DNA also exhibits a peak around 260 nm, whereas their conversion to Dewar isomers occurs most efficiently around 320 nm (3, 6). Therefore, the transmission of UV by human skin, or other factors such as the application of sun lotions which attenuate short-wave UV to a greater extent than long-wave UV, should increase the likelihood that (6–4) photoproducts will be photoisomerized into Dewar isomers.

In contrast to CDPs and (6–4) photoproducts, there is relatively little information regarding the biological effects of Dewar isomers. Earlier studies demonstrated that the survival of Streptomyces griseus spores or certain bacterial strains improved after irradiation with wavelengths between 300 and 360 nm (14, 15). This process, termed photoreactivation type III, was most efficient around 313 nm and attributed to the nonenzymatic photolysis of (6–4) photoproducts. These photolysis products were later identified as Dewar valence isomers of (6–4) photoproducts (7, 16). To account for the improved survival of S. griseus spores, it was suggested that of the two isomers, Dewar photoproducts are less cytotoxic (16). In a previous study, we have demonstrated that this appears to be true for normal human fibroblasts, but that Dewar isomers are cytotoxic to normal human T lymphocytes (5). It was also suggested that type III photoreactivation may result in the more rapid repair of UV-induced DNA damage. However, Dewar isomers and (6–4) photoproducts appear to be repaired with similar rates (17, 18). An important difference between the biological role of (6–4) photoproducts and Dewar isomers is likely to be due to their different mutagenic potential. Although the mutagenicity of Dewar isomers has not been established in mammalian cells, it has been demonstrated by transfecting single-strand DNA vectors containing a single, site-specific photoproduct into Esche-
Fig. 3. Dose-response curves for the induction of H3 (A and D), 64M-2 (B and E), and DEM-1 (C and F) antibody binding sites in human mononuclear cells following irradiation with broad spectrum FS20 UVB (A, B, and C) or natural sunlight (D, E, and F). Each data point represents the mean of three independent experiments; bars, SE. FS20 fluences were estimated by using an IL1350 radiometer with a SCS280 filter.

richia coli or Saccharomyces cerevisiae that the TC Dewar isomer is more mutagenic than the TC (6-4) photoproduct (19, 20). The TT Dewar isomer was found to be less mutagenic than the TT (6-4) photoproduct (20). Of particular note is that (6-4) photoproducts (and hence Dewar isomers via photoisomerization) form preferentially at TC sites. If, in mammalian cells, TC Dewar isomers were more mutagenic and less cytotoxic than TC (6-4) photoproducts, this would undoubtedly have important implications for the induction of mutation and skin cancers by natural sunlight.

The majority of information pertaining to the induction of UV-induced DNA damage and related biological effects has been obtained by using artificial UV sources, particularly those emitting at 254 nm. These studies have provided extremely useful information regarding the mechanisms of formation and biological effects of DNA photoproducts. However, the observations reported in this paper indicate that the spectrum of DNA damage and hence the potential for adverse effects is dependent on the spectral output of individual UV sources. Therefore, in order to understand the mechanisms behind solar UV-induced cytotoxicity, mutation, and skin cancer, the biology of individual photoproducts including Dewar isomers, as well as the interaction of the individual wave bands present in natural sunlight, should be considered. Furthermore, the ability to determine the yield of specific DNA photoproducts may make it possible to generate more meaningful risk estimates for the dangers associated with exposure to environmental UV or to artificial sources such as sunbeds.

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