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Advances in Brief

Wavelength Specific Patterns of *p53* Induction in Human Skin following Exposure to UV Radiation[1]

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Abstract

We report that, in human skin, exposure to equally erythemogenic doses of UVA, UVB, and UVC increases immunocytochemically detected *p53* in a wavelength-specific pattern. UVC produced immunostaining confined to the upper epidermis. With UVB, staining was seen throughout the epidermis, whereas with UVA staining predominated in the basal layer. The results with UVB and UVC are understandable on the basis of their known differences in penetration, whereas those with UVA are not. This suggests that within one cell type the pattern of *p53* response to UV radiation is wavelength dependent.

Introduction

Loss of function of the *p53* tumor suppressor gene by mutation or allele loss is a common finding in many human malignancies (1), including squamous cell carcinoma of the skin (2, 3). Recent studies suggest that *p53* plays an important role in regulating cell cycle progression following exposure to DNA damaging agents. In cultured normal cells the increase in *p53* observed following exposure to these agents is associated with G_1 arrest of cycling cells, allowing time for the repair of DNA damage before DNA synthesis and mitosis (4, 5). Skin cancer is the most common human malignancy and is strongly associated with exposure to UVR[3] (6). Sun-exposed human skin is continuously stressed by the DNA damaging effects of UVR. *In vitro* exposure of cultured cells to UVC increases *p53* expression (7). The UV region of the electromagnetic spectrum is subdivided into three wavebands: (*a*) UVC, 200–290 nm; (*b*) UVB, 290–320 nm; and (*c*) UVA, 320–400 nm. This classification, although defined in terms of wavelength, usefully separates UVR into three regions that have differing biological and physical properties (8). Although UVC produces skin reddening, none reaches the surface of the earth as it is efficiently absorbed by the atmosphere. UVB, although only a relatively minor component of natural sunlight, has been implicated by epidemiological and experimental studies as a major factor in the development of human skin cancer (6). UVA comprises the main UV component of natural sunlight and recent work has shown its carcinogenic potential in mice (9). Although all three types of UVR induce DNA damage, the nature and amount of damage is wavelength dependent with pyrimidine dimers of the cyclobutane type predominating at shorter wavelengths and single strand breaks and DNA protein cross-linking at longer wavelengths (10). Exposure to UV also induces other cellular responses, which are thought to serve a protective function. These include transcriptional induction of a variety of genes such as c-*jun* and c-*fos* (11, 12). For technical reasons most studies *in vitro* on the mammalian UV response have used UVC, although it is said that the pattern of response is broadly similar with other wavelengths (see references listed in Ref. 12).

The anatomical organisation of human skin and the differential penetration characteristics of UVC, UVB, and UVA mean that conclusions from *in vitro* studies may not be directly transferable to human skin since the carcinogenic potential of a particular wavelength *in vivo* will be a function of both the dose received by the stem cells in the basal layer (related to penetration characteristics) and the effectiveness of the radiation in inducing DNA damage.

Using immunocytochemistry we show that doses of UVB, UVA, and UVC which induce equivalent erythema are all capable of increasing *p53* expression, but that the pattern of expression is different for each wavelength. Furthermore the changes seen cannot be explained directly on the basis of differences in penetration.

Materials and Methods

Six healthy male volunteer members of staff aged 28–48 years were studied after informed consent had been obtained. Six sites, 10 mm in diameter, on the lower back were exposed to increasing doses of radiation at each of the 3 wavelengths (13). The following optical radiation sources were used: (*a*) UVC, germicidal lamp (Philips TUV 6W) for irradiation at 254 mm; and (*b*) UVB and UVA, an irradiation monochromator (Applied Photophysics Limited, Surrey, United Kingdom; Model UV90) optically coupled to a high pressure xenon arc lamp. The central wavelength and band width was 300 ± 5 nm for UVB and 350 ± 30 nm in conjunction with a Schott WG 335 glass filter for UVA. At 24 h after irradiation, dose-response curves were constructed for each of the wavelengths using reflectance measurements of erythemal intensity (13). From these curves, one site for each wavelength was identified that showed an increase in erythema index of 0.1, equivalent to "moderate erythema" (an increase in erythema index of 0.03 corresponds to "just perceptible erythema") (13). Biopsies were taken from the identified sites (erythema index, 0.1) for UVA, UVB, and UVC and from a control unirradiated site. Samples were fixed in formalin and paraffin embedded, and 4-μm sections were processed using DO7, a mouse monoclonal antibody against recombinant human *p53* as primary antibody (14) and a rabbit anti-mouse peroxidase conjugated secondary antibody as previously described (15). The peroxidase reaction was developed using diaminobenzidine. Results were assessed blind by two observers independently.

Results

No *p53* immunostaining was observed in unirradiated skin. Biopsies of UVR-irradiated skin showed positive nuclear signal in all cases (18 of 18). Samples showed wavelength-specific patterns of immunostaining (Fig. 1). UVC induced staining was confined to the upper layers of the epidermis (granular and upper stratum spinosum); with UVB the immunostaining was diffuse and intense throughout the epidermis with no evidence of a gradient effect with increasing depth. UVA showed a distinct pattern with immunostaining predominating in or confined to the basal layer. In one individual some immunopositive cells above the basal layer were seen with UVA, and occasional positive cells below the upper spinous cell layer were seen with UVC. However, the pattern of staining was sufficiently distinctive to allow

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[3] The abbreviation used is: UVR, UV radiation.

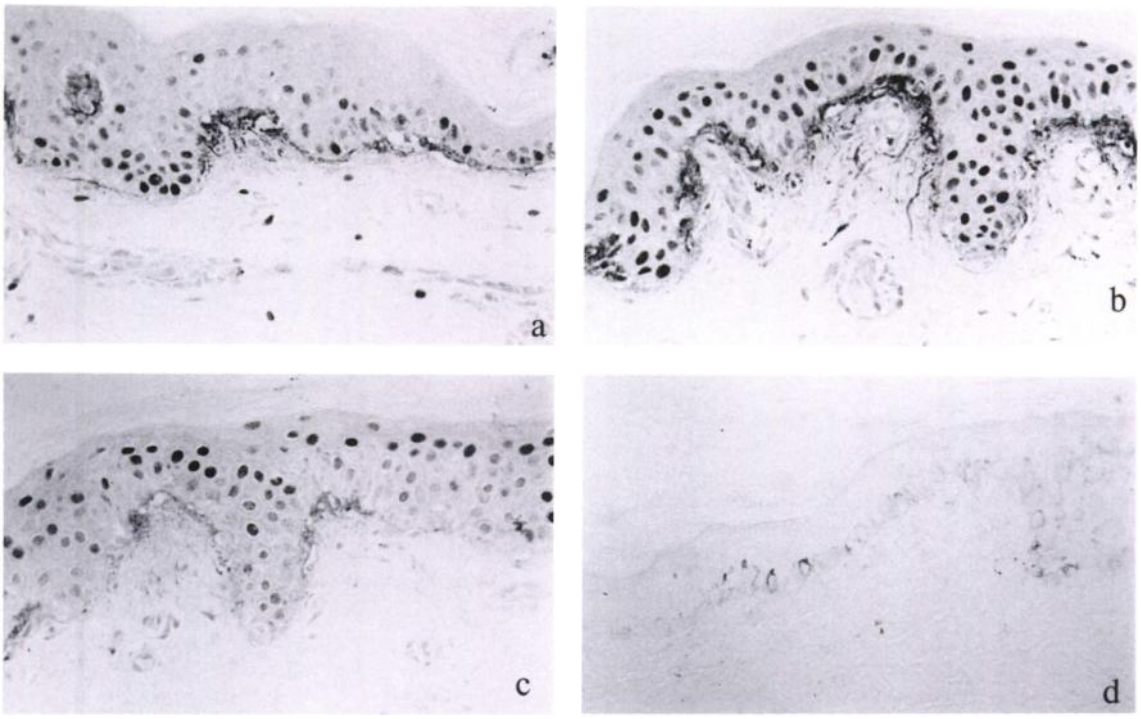

Fig. 1. *p53* immunostaining in skin irradiated with UVA (*a*), UVB (*b*), UVC (*c*), and unirradiated skin (*d*).

prediction of the wavelength of UVR used in all samples (18 of 18) based on "blind" examination of the slides by two independent observers. Immunostaining was seen in dermal fibroblasts in some individuals with UVA but not UVB or UVC. In some sections, positive immunostaining was seen at the bottom of the rete pegs (where it tended to be strongest) but adjacent fibroblasts were not stained, suggesting either differential sensitivity of the cell types or attenuating effects of the basement membrane. In general, staining obtained with UVA was less intense than that seen with UVB and UVC.

Discussion

We have shown that exposure of human skin to equally erythemogenic doses of UVC, UVB, and UVA increases *p53* expression in a wavelength-specific pattern. The observed differences between the staining pattern observed with UVC and UVB are understandable on the basis of known differences in penetration (16, 17). The pattern of epidermal immunostaining seen after UVA with basal staining predominant cannot be explained directly on the basis of differences in penetration. While this result is compatible with the ability of significant amounts of UVA to penetrate to this depth, it is surprising that the suprabasal cells showed no or little immunostaining, since they would have received a larger dose of radiation. Possible explanations for this are that (*a*) the dose of UVA above the basal layer was so great that the cells were unable to upregulate *p53* as an appropriate adaptive response; or (*b*) the mechanism of response is different from that seen with UVB and UVC, and/or some characteristic of the basal cells accounts for the specificity of the change. We think *a* is unlikely because there was little evidence of different morphological cellular damage between the wavelengths studied and because transmission characteristics of UVR through the epidermis mean that there is less variation in the dose received between basal and granular layers for UVA than for UVB or UVC (17). Furthermore, the response seen with UVB throughout the epidermis shows that up-regulation of *p53* can occur even when the absolute doses of UVB vary by a factor of six (the dose received by the basal layer will only be approximately 16% of that received by the granular layer). Explanation *b* seems more likely. This suggests that the mechanism of response with UVA is different from that seen with UVB and UVC and that some characteristic of the basal cells accounts for the specificity of the changes. Our findings of increased *p53* expression following UVR exposure confirm the observations recently reported by Hall *et al.*, who using solar simulated radiation showed *p53* immunoreactivity (using a variety of monoclonal and polyclonal antibodies) predominantly in the basal and immediately suprabasal keratinocytes and in occasional dermal fibroblasts (18). This pattern is similar to that observed by us using UVA.

The cellular mechanisms involved in the stabilization of p53 protein following UVR exposure are poorly understood. Although the observation that some other DNA-damaging agents induce a similar response suggests an important role for DNA damage in increasing *p53* expression, other pathways may play a role, such as those recently described in mediating the effects of UVR on c-*jun* expression (11). Increase in *p53* levels may reflect direct DNA damage or the ability of the cell to detect DNA damage, or both. Our results show that although the pattern of *p53* immunostaining with UVB and UVC is predictable on the basis of the known differences in penetration, the result with UVA is not. Although UVA is 1000 times less effective in inducing erythema than UVB, the 100-fold excess of UVA in terrestrial sunlight, together with the ability to selectively block out UVB with a variety of commercial sunscreens, means that recreational exposure to UVA may increase significantly. We suggest that methods comparing DNA damage or response to DNA damage secondary to UVR need to take into account both differences in biological response within the epidermis and the penetrating characteristics of the different wavelengths of UVR. Results obtained from *in vitro* studies may need to be interpreted cautiously.

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