Dissociation of Erythema and p53 Protein Expression in Human Skin Following UVB Irradiation, and Induction of p53 Protein and mRNA Following Application of Skin Irritants

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The mechanisms mediating the varied effects of ultraviolet radiation (UVR) on human skin are unclear, although a relationship between erythema and DNA damage is suggested by photosensitivity in xeroderma pigmentosum. Increased p53 expression in response to UVR is thought to reflect direct DNA damage, but recent evidence indicates that UVR also activates membrane and cytosolic signal transduction pathways. In this study, we have investigated the relationship between erythema and p53 induction following UVB and whether this p53 response is specific to UVR. p53 protein expression was determined by immunocytochemistry using the monoclonal antibody DO7, and p53 mRNA expression was examined by non-isotopic in situ hybridization. Incremental doses of UVB were administered to the lower back of eight subjects. Immunostaining revealed that p53 positive nuclei were significantly increased 8 h after suberythemogenic doses of UVB (79 ± 12), compared to normal unirradiated skin (8 ± 6, p < 0.0005), but no change in p53 mRNA was seen. Higher UVB doses, which resulted in moderate erythema, resulted in a similar or greater induction of p53 protein. Indomethacin (1% w/v), applied immediately after UVB irradiation, significantly inhibited UVB erythema at 8 h in six subjects (p < 0.005), but did not reduce p53 immunostaining. Dithranol (1 µg/µl, n = 8), sodium dodecylsulphate (5%, n = 4), and retinoic acid (0.5%, n = 4), applied for 48 h, caused erythema, significantly increased p53 protein levels (p < 0.05), and also increased p53 mRNA. Our results show that in human skin, UVB-induced p53 elevation can be dissociated from erythema and skin irritants can also induce p53 protein. The induction of p53 mRNA by irritants but not UVR suggests different mechanisms of action. Keywords: immunocytochemistry/in situ hybridization/Western blotting/dithranol. J Invest Dermatol 103:493–499, 1994

UVB exerts a variety of effects on cells and human skin including acute and delayed erythema, immediate and delayed tanning, and proliferation responses [7]. Increased cumulative exposure to UVB is associated with an increased incidence of non-melanoma skin cancer (NMSC) [8]. Induction of p53 expression in normal human skin in vivo following exposure to simulated solar radiation was first reported by Hall et al [9]. Subsequently, the pattern of p53 induction in vivo in human skin was shown to be wavelength specific [10]. UVB can cause DNA photoproducts in the p53 gene, e.g., cyclobutane dimers and 6-4 photoproducts, both at codons where mutations have not been identified [11]. Somatic mutations in the p53 tumor suppressor gene specific to UVR, e.g., CC → TT double base transitions, have been identified in NMSCs, as have other mutations, e.g., C → T base substitutions, which are likely to be due to UVR [12,13]. The mechanism of p53 induction by UVR is poorly understood but probably relates to post-translational stabilization [4,5] and although this may be due to a direct DNA damaging effect of UVB, other signal-transduction pathways may also be involved. Protein kinase C (PKC) has recently been implicated in the pathways involved in induction of p53 post-ionizing and UVB radiation [14,15]. UVB can cause lipid peroxidation of liposomal membranes and lipid peroxidation products have been shown to increase in human skin with increasing exposure to UVB [16,17]. Devary et al have identified the activation of Src tyrosine kinases as the earliest
detectable step in the induction of c-jun by UVR and thus suggested that the UV response is initiated by oxidative stress at or near the plasma membrane, rather than in the nucleus [18]. It was subsequently shown that induction of NFκB following UVR, as part of the UV response, occurs in nucleated cells and, therefore, does not require a signal generated in the nucleus [19].

In human skin, UVR-induced erythema may be quantified by the use of a reflectance instrument and wavelength-specific dose-response curves have been observed [20]. Whether a direct relationship between erythema and DNA damage exists is unclear, but this is suggested by the increased photosensitivity observed in xeroderma pigmentosum patients [21]. On the other hand, the pathways mediating the initial stages of acute UVR-induced inflammation involve arachidonic acid release from membrane phospholipids and its subsequent metabolism via the cyclooxygenase system to prostaglandins [22,23]. Topical indomethacin (a potent cyclooxygenase inhibitor) has been shown to significantly reduce acute UBV-induced erythema [24,25]. In this study, we have investigated the relationship between acute UBV erythema and p53 protein and mRNA levels to address whether signal-transduction pathways involved in the acute erythema response are also involved in the induction of p53. We have also addressed whether other irritants that cause erythema in skin induce p53 expression.

MATERIALS AND METHODS

Subjects Investigations were carried out on the healthy skin of the lower back of 24 adult volunteers (17 male, 7 female) ages 21 to 81 years, mean age 57 years. All volunteers gave written informed consent. Exclusion criteria included a past history of photosensitivity and any regular oral medication.

UVR Irradiation The UBV source was an irradiation monochromator (Applied Photophysics Limited, Surrey, UK, Model UV900) optically coupled to a high-pressure xenon arc lamp, with a central wavelength of 300 nm and half-bandwidth of 45 nm. The diameter of the beam was 10 mm.

Twelve doses of UBV (2.5-112 J/cm², 2 increments) were administered to the lower back of eight volunteers. At 8 h, erythema intensities were measured using a reflectance instrument (IC Imaging, High Wycombe, UK) and a visual assessment of the minimal erythemal dose (MED) was also made. UBV-induced erythema is maximal between 8 and 12 h post-irradiation [26,27]. Biopsies were taken from normal unirradiated skin (n = 8), UBV-irradiated skin with no visually perceptible erythema (sub-MED, n = 8), and UBV-irradiated skin with an erythema index nearest to 0.1 above baseline, equivalent to moderate erythema (n = 4).

Two series of seven doses of UBV (14-112 J/cm², 2 increments) were administered to the lower back of six volunteers. Immediately after irradiation, indomethacin (1% w/v) was applied, under occlusion, at a surface density of 200 µg/cm², to one set of incremental doses and the gel base applied, under occlusion, to the other set. The occlusive patches were removed at 2 h and any residual topical agent allowed to dry. At 8 h, erythema intensities were recorded using a reflectance instrument. The gel site that gave an erythema index nearest to 0.1 above baseline and the indomethacin site that had received the same dose of UBV were identified and biopsies taken from these two sites.

Application of Irritants Twenty-microliter aliquots of a) dithranol (1 µg/µl) in chloroform, b) sodium dodecyl sulphate (SDS) (5%) in propylene glycol/ethanol (30:70), c) retinoic acid (RA) (0.5%) in propylene glycol/ethanol (30:70), and d) propylene glycol in ethanol (30:70) (PG) were applied, using Finn chambers, to the skin of the lower back of four volunteers. Finn chambers were removed at 48 h; erythema was assessed at 20 min later using a reflectance instrument and biopsies taken from each of the four sites. In four other volunteers, dithranol (1 µg/µl) in chloroform, chloroform, and PG, and a dry filter disk were applied under Finn chambers for 48 h and in two additional volunteers dithranol, SDS, RA, and a dry filter disk were applied as above for 8 h. Erythema indices were assessed and biopsies performed at these time points.

Biopsy Procedure Four-millimeter punch biopsies were performed under local anesthesia, immediately fixed in paraformaldehyde for 3 h and embedded in paraffin. Samples from the same individual were embedded in the one paraffin block to minimize variation during immunocytochemical and in situ hybridization procedures.

Immunocytochemistry Four-micrometer sections were immunostained with DO7 (a mouse monoclonal antibody against recombinant human p53; Novocastra Laboratories Ltd., Newcastle, UK), for 30 min at room temperature, after prior incubation for 10 min with normal rabbit serum. Sections were stained both with and without prior antigen retrieval by microwaving the sections for 10 min in sodium citrate buffer (pH 6.0) in a 600-watt conventional microwave oven [28]. After incubating for 30 min with a biotinylated rabbit anti-mouse antibody, a streptavidin-biotin-peroxidase detection system (Dako) was employed with diaminobenzidine (DAB) as the substrate. The number of positively stained epidermal nuclei in four high-power fields (HPF) was assessed by an independent observer who was unaware of the treatment conditions. Hematoxylin and eosin staining was also performed on all biopsy specimens.

Western Blotting Biopsies were finely minced, frozen in liquid N₂, and ground to a fine powder. Protein extraction buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 5 mM ethylenediaminetetraacetic acid, 1% NP40, 1 mM phenylmethylsulfonyl fluoride) was added. Following sonication for 1 min, samples were left on ice for 30 min, centrifuged at 15,000 × g for 30 min and the supernatant (protein) transferred to a fresh tube. Samples were stored at −20°C until required. Protein concentration was measured spectrophotometrically (Pierce BCA Protein Assay Reagen). Three to five micrograms of total protein was separated by gel electrophoresis (5% stacking gel, 10% running gel) and blotted onto nitrocellulose membrane. Membranes were blocked for 1 h at room temperature with 5% nonfat milk/0.2% Tween 20 and incubated sequentially with DO7 supernatant (5-25 µg/ml) and rabbit anti-mouse peroxidase-conjugated secondary antibody. The peroxidase reaction was developed using ECL (Amersham). A colonic carcinoma cell line (HT29) with a p53 mutation at codon 273, causing an Arg → His substitution, served as a positive control [29].

Keratinocyte Cultures Human foreskin keratinocytes were isolated as described [30] and were grown in serum-free keratinocyte medium ( Gibco) at 37°C in a 95% air/5% CO₂ humidified incubator. Cells at passage 2 were irradiated for 3 min with a broadband UVB source [31] (Wolf Helium Bal 1-0140, West, Cosmedico, Stuttgart, Germany) equivalent to a UVB dose of 87 mJ/cm², and protein was extracted 3 h later using protein extraction buffer as described above.

Probe Preparation Antisense and sense digoxigenin-labeled cRNA transcripts were generated from the 1800 base pair (bp) p53 cDNA clone pBSK-SN3 [22] using a Digoxigenin RNA Labeling Kit (Boehringer Mannheim), according to the manufacturer’s instructions. Full-length transcripts were hydrolyzed to average lengths of 300 bp, prior to recovery by ethanol precipitation.

In Situ Hybridization Four-micrometer paraffin sections were subject to in situ hybridization as previously described [33]. Briefly, sections were pretreated with 5 µg/ml proteinase K (Boehringer Mannheim)/0.1 M Tris-HCl, pH 8.0, 50 mM ethylenediaminetetraacetic acid, at 37°C for 15 min, immersed in 0.2% glycine for 5 min, and rinsed in 300 mM NaCl, 30 mM sodium citrate, 0.1% (w/v) SDS at 52°C. Following prehybridization (60 min at 42°C), 30 µl of hybridization buffer (4 X SSC, 1 X Denhardt’s solution, 50% deionized formamide, and 100 µg/ml of each of denatured salmon sperm DNA and Escherichia coli transfer RNA) containing 20 ng of digoxigenin-labeled cRNA probe was applied to each section. Adjacent sections were hybridized with antisense and sense probes. Following hybridization, slides were washed to a final stringency of 0.1 X SSC/50% formamide at 45°C. Following a 2 X SSC rinse, the tissue was dehydrated stepwise, autoradiographed, and detected using a Digoxigenin Nucleic Acid Detection Kit (Boehringer Mannheim), according to the manufacturer’s instructions. Slides were monitored for colorimetric development, which was stopped in TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid; pH 7.4).

Statistics Summary statistics are reported as means ± SEM. Comparisons between means were performed using unpaired or paired t tests as appropriate. The data was analyzed using microstat software (Ecosoft Inc).

RESULTS

Effect of UBV Irradiation on p53 Protein Expression in Human Skin p53 protein expression was assessed by immunocytochemistry in skin 8 h following irradiation with erythemogenic and suberythemogenic doses of UBV. Doses of UBV received by the sub-MED biopsy sites ranged from 10 to 40 mJ/cm² (MED 14-56 mJ/cm²) (Table I). The absolute erythema indices correlated with the absence of visible erythema, with no significant difference between normal and sub-MED erythema indices (p = 0.36). The number of p53-positive nuclei/HPF was significantly increased in sub-MED skin (79 ± 12) compared to normal unirradiated skin (8 ± 6, p = 0.0003) (Figs 1 and 2). p53 staining was entirely nuclear with no cytoplasmic staining and was evenly dis...
tributed throughout the epidermis, in all cases, following UVB irradiation. Fibroblasts in the upper dermis also stained positive in most cases following irradiation. Prior microwaving of tissue sections caused increased intensity of positive staining and gave slightly higher counts per HPF in irradiated cases, but did not alter the low numbers of positively stained nuclei in unirradiated skin. Doses of UVB sufficient to cause an increase in the erythema index of 0.1 above baseline in four volunteers ranged from 28 to 80 mJ/cm². Two patterns of p53 induction were observed in the biopsies of sub-MED and moderately erythematous skin in these four subjects (Table I). In two volunteers, the number of p53-positive nuclei/HPF in both the sub-MED skin (53 and 117) and the irradiated skin with an erythema index of 0.1 above baseline (62 and 120, respectively) were similar. In two other subjects, the number of p53 nuclei/HPF increased in proportion to the UVB dose received (122 at 80 mJ/cm² and 220 at 80 mJ/cm² for one subject and 25 at 10 mJ/cm² and 128 at 28 mJ/cm² for the other).

To ensure that the increase in p53 immunostaining in response to UVR was due to increased p53 protein, we performed Western blotting. Figure 3 demonstrates an increase in the intensity of the 53-kDa (p53) band following UVB irradiation of human skin and cultured human keratinocytes.

**Table I. Summary of p53 Protein and p53 mRNA Expression in UVB-Irradiated Subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/Sex</th>
<th>MED (mJ/cm²)</th>
<th>SubMED (mJ/cm²)</th>
<th>0.1 EI (mJ/cm²)</th>
<th>p53 Protein (nuclei/HPF) and mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73/F</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 5/+ SubMED 66/+ 0.1 EI 167/+</td>
</tr>
<tr>
<td>2</td>
<td>68/F</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 48/+ SubMED 102/+ 0.1 EI 167/+</td>
</tr>
<tr>
<td>3</td>
<td>21/F</td>
<td>56</td>
<td>40</td>
<td>0.1</td>
<td>Normal 1/+ SubMED 58/+ 0.1 EI 94/+</td>
</tr>
<tr>
<td>4</td>
<td>73/F</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 5/+ SubMED 89/+ 0.1 EI 107/+</td>
</tr>
<tr>
<td>5</td>
<td>63/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 0/+ SubMED 117/+ 0.1 EI 53/+</td>
</tr>
<tr>
<td>6</td>
<td>22/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 0/+ SubMED 53/+ 0.1 EI 62/+</td>
</tr>
<tr>
<td>7</td>
<td>52/M</td>
<td>14</td>
<td>10</td>
<td>0.1</td>
<td>Normal 0/+ SubMED 25/+ 0.1 EI 138/+</td>
</tr>
<tr>
<td>8</td>
<td>21/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 0/+ SubMED 25/+ 0.1 EI 138/+</td>
</tr>
<tr>
<td>9</td>
<td>63/F</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 2/+ SubMED 25/+ 0.1 EI 138/+</td>
</tr>
<tr>
<td>10</td>
<td>40/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 2/+ SubMED 25/+ 0.1 EI 138/+</td>
</tr>
<tr>
<td>11</td>
<td>70/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 122/ND SubMED 220/ND 0.1 EI 112/+</td>
</tr>
<tr>
<td>12</td>
<td>46/F</td>
<td>112</td>
<td>14</td>
<td>0.1</td>
<td>Normal 94/+ SubMED 107/+ 0.1 EI 112/+</td>
</tr>
<tr>
<td>13</td>
<td>38/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 53/+ SubMED 56/+ 0.1 EI 38/+</td>
</tr>
<tr>
<td>14</td>
<td>76/M</td>
<td>28</td>
<td>20</td>
<td>0.1</td>
<td>Normal 195/+ SubMED 196/+ 0.1 EI 195/+</td>
</tr>
</tbody>
</table>

* Levels of expression of p53 protein (positive nuclei per high power field) and p53 mRNA (graded +/++/+++). ND indicates not done.

Effect of UVB Irradiation on p53 mRNA Expression in Human Skin In *in situ* hybridization, using a digoxigenin-labeled antisense p53 cRNA probe, showed positive cytoplasmic signal in the lower layers of the suprabasal epidermis of normal unirradiated skin, with 10–20% of basal cells also being positive. At 8 h post-UVB irradiation a small increase in the number of p53 in *in situ* hybridization positive basal cells was observed in skin with an erythema index of 0.1 above baseline, whereas no changes were identified in the sub-MED biopsies (Table I). No increase in the signal was seen in the suprabasal compartment post-irradiation in spite of the changes in immunocytochemistry. For each sample, specificity of hybridization was confirmed using sense-strand and unrelated probe (keratin 14) controls, as previously described [33].

Effect of Indomethacin on UVB-Induced p53 Protein Expression in Human Skin We next examined whether inhibition of UVB-erythema affects p53 expression in human skin. Doses of UVB sufficient to cause an increased erythema index of 0.1 above baseline at 8 h ranged from 14 to 40 mJ/cm² in six volunteers (Table I). Indomethacin significantly inhibited UVB-erythema compared to control (75 ± 16% reduction, p = 0.0014). Positive p53 immunostaining was observed in all 12 biopsies at 8 h post-irradiation. The mean number of p53-positive nuclei/HPF was 110 ± 25 for the UVB/gel-treated skin and 114 ± 26 for the UVB/indomethacin-treated skin. Thus although indomethacin significantly inhibited UVB erythema, indomethacin failed to inhibit induction of p53 by UVB (Fig 4). No significant change in p53 mRNA level and distribution was identified following the application of indomethacin.

Effect of Irritants on p53 Protein Expression in Human Skin We then investigated whether other erythemogenic agents also induce p53 expression in human skin. At 48 h after the application of irritants, epidermal nuclear p53 protein was detected in all irritant biopsy specimens. The mean number of p53-positive nuclei per HPF was 46 ± 14 for dithranol, 59 ± 29 for SDS, 55 ± 31 for RA, 32 ± 13 for PG, 36 ± 12 for chloroform, and 17 ± 25 for normal skin. The pattern of p53 induction was evenly distributed throughout the epidermis for all irritants but individuals showed different sensitivities to the different substances. The increase in p53-positive nuclei/HPF was significant for dithranol, SDS, and RA compared to normal untreated skin (p < 0.05). In general, the intensity of staining was greater in UVB-treated skin, even at sub-MED doses, than in irritant-treated skin. The mean increase in the erythema indices were 0.192 ± 0.034 for dithranol, 0.121 ± 0.025 for SDS, 0.054 ± 0.017 for RA, 0.099 ± 0.004 for PG, and 0.034 ± 0.012 for chloroform. No relationship was detected between the number of p53-positive nuclei per HPF and the increase in erythema index following the application of irritants.

![Figure 1. Dissociation of erythema and p53 protein expression in human skin following UVB irradiation. a) Erythema index and b) number of p53 immuno-positive epidermal nuclei per HPF in normal skin and 8 h after UVB irradiation (SubMED, skin with no detectable erythema that had received one dose below the MED; 0.1 EI, skin that had received dose sufficient to increase the erythema index 0.1 above baseline). Immunostaining using DO7 anti-p53 antibody was carried out as described in Materials and Methods. All values are means ± SEM, * n = 4, *n = 8.](image-url)
Figure 2. Increased p53 immunostaining following suberythmogenic and moderately erythmogenic doses of UVB in human skin. Irradiation of human skin was carried out as in Fig 1. Immunostaining using DO7 anti-p53 antibody was carried out as described in Materials and Methods. a) Normal unirradiated skin, b) SubMED, and c) 0.1 EI from one subject. Scale bars, 12.5 μm.

In the two volunteers biopsied at 8 h after application of the irritants, an increase in the number of epidermal p53-positive nuclei per HPF was observed in dithranol-treated skin (46 and 40) as compared with normal occluded skin (9 and 0) (Fig 5a,b). An increase was also observed in SDS- and RA-treated skin at 8 h in one volunteer (69 : 49 : 9 for SDS : RA : normal occluded) but there was no p53 protein detected in the corresponding biopsies from the second volunteer.

Effect of Irritants on p53 mRNA in Human Skin As compared with normal untreated epidermis, a large increase in the intensity of signal and the number of both basal and suprabasal positive cells, as detected by in situ hybridization, was observed at 8 and 48 h following application of dithranol, SDS, and RA. Individuals varied in their sensitivity to these irritants although dithranol caused a consistent increase in p53 mRNA in all cases (Fig 5c,d).

DISCUSSION

p53 is a tumor suppressor gene, functioning as a “guardian of the genome” by causing cells to arrest in late G1 following exposure to energy-rich radiation, e.g., γ-radiation [34]. The high incidence of mutations of this gene in NMSC suggests that it plays an important physiologic role in the skin [12,13], and the induction of wild-type p53 in human epidermis in response to UVR is likely to be relevant in the prevention of skin carcinogenesis. Although DNA damage by cytotoxic agents, e.g., cisplatin, mitomycin C, etoposide, and actinomycin D, causes post-translational stabilization of p53 protein [5], the site of initiation of p53 induction by UVR is not well established. UVR causes a wide variety of responses including erythema [7]. We therefore investigated whether a relationship existed between erythema and p53 induction in human skin following UBV.

Figure 3. Western blot analysis of p53 protein expression in (A) human skin and (B) cultured human keratinocytes. Western blotting using DO7 anti-p53 antibody was performed as described in Materials and Methods. A) M, markers (ECL, Amersham), from top to bottom 97.4, 68, 46, 31, 20.1, and 14.4 kDa. Lane 1, human skin at 8 h after irradiation with moderately erythmogenic dose of UVB (112 mJ/cm²); lane 2, normal unirradiated human skin. B) Lane 1, normal unirradiated cultured human keratinocytes; lane 2, cultured human keratinocytes at 3 h after irradiation with 3 min of a broad band UVB source [31] (Wolff Helarium B1-01-40W, Kosmedico, Stuttgart, Germany) equivalent to a UVB dose of 87 mJ/cm²; C, positive control (HT29 colonic carcinoma cell line).

Figure 4. UVB-induced erythema but not p53 protein expression is reduced by topical indomethacin in human skin. Irradiation of human skin was carried out as in Fig 1, with application of indomethacin or gel immediately after irradiation. Immunostaining using DO7 anti-p53 antibody was carried out as described in Materials and Methods. a) Increase in erythema index above baseline and b) number of p53 immune-positive epidermal nuclei per HPF, following doses of UVB sufficient to cause an increase in the erythema index of 0.1 above baseline at the gel site (UVB/gel) with same doses applied to the indomethacin site (UVB/indo). Values are means ± SEM, n = 6.
and whether other erythemogenic agents were capable of inducing p53.

The erythema response is widely used as a measure of UV exposure and indirectly as a risk factor for NMSC in human skin [35]. Hall et al demonstrated an increase in p53 protein levels using doses of solar simulated radiation sufficient to cause erythema equivalent to 1.5 MEDs [9]. Campbell et al compared equi-moderately erythemogenic doses of UVA, UVB, and UVC, and demonstrated a wavelength-specific pattern of p53 induction [10]. A large increase in p53 protein levels in human skin post-UVB irradiation, at a dose sufficient to cause an increase in the erythema index of 0.1, was observed in this study using both immunocytochemistry and Western blotting. Thus, Western blotting confirmed that the DO7 antibody binds to increased amounts to p53 protein following UVB irradiation and also shows that the increased immunocytochemistry staining observed is not due to non-specific binding of the antibody to another antigen that might have become altered or unmasked after exposure to UVB. However, as our results show, doses of UVB that do not cause erythema (both on visual and reflectance instrument assessment), cause a significant increase in p53 protein levels in human epidermal cells. At these suberythemogenic doses of UVB the pattern of induction of p53 protein was evenly distributed throughout the epidermis as in the case of moderately erythemogenic doses. If DNA injury is a prerequisite for induction of p53, these results suggest that significant epidermal nuclear damage occurs at low doses of UVB and highlight the importance of physi-
ologic non-erythemogenic doses of UVB, i.e., doses of UVB below that which causes sunburn, in skin carcinogenesis. Although low doses of UVC are capable of causing photoproducts in the p53 gene in vitro [11], whether low sub-erythemogenic doses of UVB can cause similar changes in the p53 gene in vivo is, at present, unknown.

Interestingly, in two of the four subjects in whom biopsies were taken from subMED and moderately erythematous skin, the number of p53-positive nuclei per HPF was similar for both doses of UVB. In the other two cases, a greater number of p53-positive epidermal nuclei were identified at the higher UVB dose. This inter-subject variation may be important in the protection offered to the cell against the subsequent development of neoplasia, and therefore for individual risk. The reason for p53 upregulation in the cycling cells of the basal and lower suprabasal layers is obvious in this regard, but not so obvious for cells in the upper stratum spinosum. The participation of p53 in the initiation of apoptosis might be more relevant here, yet hematoxylin and eosin staining showed only one to two apoptotic cells/HPF in skin biopsies with an erythema index of 0.1 above baseline in contrast to the dramatic increase in the number of p53-positive nuclei throughout the epidermis.

The acute erythema response following UVB involves release of arachidonic acid from the cell membrane and its subsequent metabolism by the cyclo-oxygenase pathway [22,23]. Indomethacin is a potent inhibitor of cyclooxygenase and has been shown to cause significant suppression of acute-phase UVB-induced erythema when applied topically to human skin [24,25]. Arachidonic acid can stimulate activation of the PKC signal-transduction system, and activation of PKC further increases the release of arachidonic acid from phospholipids [36]. TPA and calphostin C modulate the induction of p53 in murine fibroblasts and ataxia-telangiectasia cells respectively following ionizing and UVB radiation, suggesting that PKC may be involved in this process [14,15]. In this study, topical indomethacin significantly inhibited UVB-induced erythema, but failed to alter UVB induction of p53 protein, suggesting that products of the cyclo-oxygenase pathway are not involved in p53 induction post-UVB. These data together with our observation of p53 induction with suberythemogenic doses of UVB indicate that increased p53 expression following UVB irradiation may be dissociated from UVB-induced erythema and suggests that these events are mediated by different signal-transduction pathways.

The mechanism of increased p53 protein expression after UVR was originally investigated by Maltzman and Czyzyk using UV-C in non-transformed murine fibroblasts and was shown to be, in part, due to post-translational stabilization [4]. Fritsche et al confirmed increased p53 protein stability following UVR but also demonstrated ongoing p53 translation [5]. Smith and Rees have demonstrated that p53 mRNA levels in human skin do not differ from baseline at 24 and 48 h after UVR [33]. Our results using in situ hybridization show no increase in p53 mRNA levels in the suprabasal epidermis and minor increases in the signal in the basal epidermis at 8 h and provide further evidence that induction of p53 protein in human skin post-UVB occurs predominantly at a post-transcriptional stage.

The significant increase in p53 protein levels in human skin observed following application of dithanol, SDS, and RA suggest that up-regulation of p53 may be a non-specific response of keratinocytes to noxious stimuli. Although all these agents have the ability to cause skin irritation, they differ in the intracellular pathways through which their effects are mediated and may differ in their mechanism of p53 mRNA induction. Although dithanol may cause DNA damage via oxidative stress [37], and RA can act indirectly on DNA via members of the retinoic acid receptor (RAR) and RXR families to effect gene transcription [38,39], the mechanism of p53 induction by SDS is unclear. PKC phosphorylates p53 protein [40], and modulates the induction of p53 after ionizing and UVB radiation [14,15], and dithanol is known to inhibit PKC in vitro [41]. Thus, it is possible that activation of cell membrane and cytosolic signal-transduction pathways may be responsible for the p53 induction observed with skin irritants. The fact that no relationship was detected between the number of p53-positive nuclei/HPF and the increase in erythema index following the application of these irritants further confirms that products of the cyclooxygenase pathway are unlikely to be involved in p53 protein induction, but does not rule out the possible involvement of other cell membrane events.

Our in situ hybridization results on irritant-treated skin showed an increase in p53 mRNA at both 8 and 48 h post-application and are therefore of particular interest. This result is in contrast to the absence of p53 mRNA induction following UVR and shows that different mechanisms may account for increasing p53 protein in vivo in response to various stimuli. That different mechanisms operate in vivo to upregulate p53 protein suggests that this induction is not merely a non-specific response of keratinocytes to all noxious stimuli.


