The primary function of the epidermis is to provide a protective barrier against numerous environmental insults, including ultraviolet radiation (UVR). UVR, particularly in the UVB spectrum, is a potent carcinogen known to damage DNA directly or through the generation of free radicals. Although in the long term, protective measures such as apoptosis and inflammation may prove beneficial in safeguarding the epidermis against the propagation of potentially tumorigenic cells, after high-dose UV irradiation these biologic events may be acutely detrimental to the architectural and functional integrity of the tissue owing to rampant cell death and inflammatory responses, which can culminate in epidermal erosion and consequently loss of barrier functions. The mitogen-activated protein kinase (MAPK) signaling pathway is known to be activated by UVR and herein we identify p38 MAPK as a key modulator of these physiologic events. Mice treated with the p38 MAPK inhibitor SB202190 are protected against several detrimental effects of acute UV irradiation, namely, sunburn cell/apoptosis, inflammation, and a hyperproliferation response. Based on our results, selectively blocking p38 activation with the SB202190 inhibitor could prove beneficial in treating victims from severe sunburn or exposure to other chemical agents known to trigger the p38 pathway.

Key words: keratinocyte/sunburn cell/inflammation/p38 MAPK/UV.

Ultraviolet radiation (UVR) is a potent damaging agent that continuously challenges the epidermis. UVB radiation in particular constitutes the major contributor to many adverse cutaneous effects such as erythema, edema, blistering, hyperplasia, and nonmelanoma skin cancer among others (Afaq and Mukhtar, 2001). UVB radiation exposure is known to induce a wide variety of cellular damage to epidermal keratinocytes including DNA damage, oxidative stress, and immunotoxicity (Sarasin, 1999; Cadet et al, 2001; Clydesdale et al, 2001; Wenk et al, 2001). UBV radiation will directly and indirectly target the DNA, producing a variety of lesions, such as cyclobutane pyrimidine dimers, pyrimidine-pyrimidine (6-4)photoproducts and oxidative DNA base damage, which are believed to be the initiating steps in tumorigenesis (Cadet et al, 2001). Therefore, substantial UVR exposure may lead to detrimental acute effects such as tissue damage owing in part to the organism’s surveillance mechanism(s) attempting to eliminate damaged cells through apoptosis and inflammation (Cadet et al, 2001; Clydesdale et al, 2001).

A landmark discovery in the photobiology of the skin was the finding that apoptosis after UVR requires the p53 tumor suppressor pathway (Ziegler et al, 1994). As in human skin, UVR of normal mouse skin rapidly triggers apoptosis of keratinocytes, commonly referred to as “sunburn cells,” and a sunburn phenotype. In contrast, mice deficient in this pathway, such as Trp53−/− (p53 knockout), show markedly reduced apoptosis and sunburning response (Ziegler et al, 1994), p53’s “guardian” role in preventing tumorigenesis is due in large part to its ability to regulate the expression of multiple stress-inducible genes whose products are involved in maintaining genomic integrity such as cell-cycle regulation, DNA repair, and apoptosis (Amundson et al, 1998). Although the ATM pathway has a prominent role in p53 activation after ionizing radiation, p53 activation after UVR has important differences (Zhan et al, 1993; Bulavin et al, 1999; Appella and Anderson, 2000) in that signaling is typically not through ATM but rather other pathways such as the mitogen-activated protein kinase (MAPK) signaling pathway (Bulavin et al, 1999; Wang et al, 2000; Hildesheim et al, 2002). In particular, the p38 portion of the MAPK signaling pathway has been shown to have a major role in p53 activation after UVR, particularly of its proapoptotic functions (Bulavin et al, 1999; Takekawa et al, 2000). Additionally, p53-effector gene products, such as Gadd45a, have recently been found to be involved in a positive feedback loop modulating p38 activity, and consequently p53 activity, after UVR (Takekawa and Saito, 1998; Takekawa et al, 2000; Hildesheim et al, 2002). As a result, Gadd45a−/− mice are resistant to UVR-induced apoptosis/sunburn cells owing to a large extent to a loss of sustained...
p38 MAPK activity, and the phenotype of UV-irradiated Gadd45a−/− skin is remarkably similar to Trp53−/−.

Although p38- and p53-mediated apoptosis and inflammation are critical in protecting the integrity of the epidermis against the tumorigenic effects of UVR-induced damage, acute UVR exposure may lead to tissue damage owing to rampant apoptosis, inflammation, and necrosis (Clydesdale et al, 2001). In this report, we have studied the effectiveness of the p38/β inhibitor SB202190 in blocking p38 and p53 activity and in doing so preventing the acute responses to UVR irradiation leading to tissue erosion. We demonstrate that SB202190 is able to significantly block acute UVR-induced sunburn cells and subcorneal pustules. Our results indicate that p38 MAPK inhibitors may have utility in the management of acute solar injuries as well as injuries resulting from many chemical damaging agents that trigger the p38 MAPK pathway.

Results

p38 inhibitor blocks UVR-induced sunburn cells UVR-damaged keratinocytes are genetically programmed to undergo apoptosis at sufficiently high doses presumably as a precaution against propagating potentially tumorigenic cells (Hildesheim et al, 2002). Because p38 MAPK is known to be activated by UVR and promote apoptosis via p53, we sought to determine whether the p38/β inhibitor SB202190 is able to block sunburn (apoptotic) cells from being generated after an acute dose of UVR radiation (Hildesheim et al, 2002). As shown in Fig 1, adult mice irradiated with 2000 Jm−2 UVR radiation are able to effectively block apoptotic sequences when treated with this inhibitor. In stark contrast, animals not treated with SB202190 develop appreciable numbers of sunburn cells. Characteristic dyskeratotic sunburn cells with pyknotic nuclei are readily detected by histologic analysis (Fig 1a) and confirmed immunohistochemically by performing the TUNEL assay (Fig 1b). The TUNEL assay selectively detects nuclei with fragmented DNA, a hallmark of apoptosis. Moreover, quantitative analysis of the data (Fig 2) demonstrates that animals treated with SB202190 have a 60% reduction in the number of sunburn cells relative to untreated irradiated controls (p = 0.016, Table I). A noticeable protective effect is also observed in mice treated with SB202190 either 30 min (46% reduction) or 120 min (23% reduction) after UVR irradiation, although as noted, the effectiveness of the inhibitor in blocking the generation of sunburn cells decreased with increased delay in administration of the inhibitor (data not shown). Unlike the dramatic inhibitory effects observed with SB202190 on the irradiated groups, the treatment of the unirradiated controls with the inhibitor had no significant effect (p = 0.062). Therefore, blocking UVR-induced p38 activation significantly blocks epidermal keratinocytes from an acute sunburn response.

UVR-induced sunburn cells are a result of p38 activation and not JNK activation Although both p38 and JNK may promote apoptosis after genotoxic stress (Robinson and Cobb, 1997; Martin-Blanco, 2000), our results indicate that sunburn cells emerge primarily as a result of p38 MAPK activation and not JNK MAPK activation. Although p38 phosphorylation is not blocked by SB202190 (Fig 3a), its activity is blocked by the inhibitor, which prevents p38 from activating its downstream target p53. p38 activity (and consequently p53 activity) is measured by the ability of keratinocytes to express the p53-effector gene product, p21, after UVR. Nuclear accumulation of p21 is only detected in basal keratinocytes of UV-irradiated mice not treated with the inhibitor (arrows, bottom left panel). In contrast, irradiated SB202190-treated mice (bottom right panel) are similar to untreated controls (top panels). Representative images of experiments performed in triplicate are shown (three animals per group). Scale bar, 20 μm.

Figure 1 Effects of the p38 inhibitor SB202190 on UVR-induced sunburn cells. Adult mice were irradiated with 2000 Jm−2 UVR, and the exposed skin was harvested 24 h after UVR for histology/immunohistochemistry after formalin fixation. (A) Hematoxylin and eosin staining reveals that epidermal keratinocytes of SB202190-treated mice (bottom right panel) are resistant to UVR-induced sunburn/apoptosis. In sharp contrast, untreated, UV-irradiated mice (bottom left panel) readily develop sunburn cells (arrows) with the characteristic dyskeratotic cytoplasm and pyknotic nuclei. The epidermises from unirradiated and SB202190-treated mice (top right panel) rarely develop apoptotic cells and is indistinguishable from unirradiated and untreated controls (top left panel). (B) TUNEL assay on the same tissues demonstrate the same effect as above, whereby DNA fragmentation is widely detected in epidermal keratinocytes of UV-irradiated mice not treated with the inhibitor (arrows, bottom left panel). In contrast, irradiated SB202190-treated mice (bottom right panel) are similar to untreated controls (top panels). Representative images of experiments performed in triplicate are shown (three animals per group). Scale bar, 20 μm.
UVR-induced inflammation and hyperproliferation are blocked by the p38 inhibitor

Additional detrimental physiologic responses to acute UVR include inflammation and epidermal hyperplasia (Clydesdale et al., 2001). Both of these effects are dramatically reduced with the p38 inhibitor treatment, irrespective of whether the inhibitor was administered 30 min before or 120 min after UV irradiation (data not shown). As shown histologically, irradiated mice rapidly develop subcorneal pustules (Fig 4, panel v) that are readily blocked by the p38 inhibitor (Fig 4, panel iv). Moreover, SB202190-treated mice do not display a hyperproliferative reaction to UV irradiation and maintain a normal thickness epidermis comparable to uninjured controls (Fig 4, panels i, ii, and iv). In contrast, UV-irradiated mice not treated with the inhibitor have a strong hyperproliferative response and develop a significantly thicker and more cellular epidermis (Fig 4, panel iii). Therefore, blocking of p38 MAPK signaling with SB202190 mitigates the acute responses and subsequent damage in the epidermis enabling the skin to maintain its normal architectural integrity.

Table I. Effect of the p38 MAPK inhibitor SB202190 on UVR-induced sunburn cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Average sunburn cells/cm²</th>
<th>p value (with or without SB202190)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With SB202190</td>
<td>Without SB202190</td>
</tr>
<tr>
<td>Control</td>
<td>1.50 ± 0.54</td>
<td>0.66 ± 0.51</td>
</tr>
<tr>
<td>UVR</td>
<td>10.83 ± 2.31</td>
<td>4.16 ± 1.47</td>
</tr>
<tr>
<td>p value (with or without UVR)</td>
<td>0.015</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Discussion

The epidermis is at the forefront of protection to the organism against multiple environmental insults. One such insult is UVR, which is considered to be a potent and complete carcinogen causing the most common form of cancers, nonmelanoma skin cancers (Quinn, 1997; Brash and Ponten, 1998; Wikonkal and Brash, 1999). Keratinocytes are well equipped to protect the integrity of the epidermis against the propagation of potentially cancerous cells that may originate after chronic exposure to UVR. The built-in responses to genotoxic stress include genetic activation of programmed cell death, activation of an inflammatory response to effectively eliminate damaged cells, and eventual replacement of ill-fated cells most likely through hyperproliferation. UVR-induced activation of the MAPK signaling pathway in keratinocytes is central to this surveillance process (Hildesheim et al., 2002). UVR-induced p38 MAPK signaling ultimately results in activation of numerous transcription factors, such as p53, c-Myc, and ATF-2, that promote expression of genes involved in regulating apoptosis, DNA repair, and cell proliferation (Bulavin et al., 1999; Cross et al., 2000; Martin-Blanco, 2000; Hildesheim et al., 2002). Contrary to the effectiveness of these mechanisms under most circumstances, activation of the stress and proinflammatory cytokine-inducible p38α and p38β MAPK after acute UVR injury may result in severe destruction of the skin owing to overwhelming apoptosis, inflammation, and necrosis among other effects (Afaq and Mukhtar, 2001; Clydesdale et al., 2001). Keratinocytes are known to express proinflammatory cytokines after UVR, such as interleukin-1, interleukin-6, and tumor necrosis factor-α (Abeyama et al., 2000). Combined, UVR-induced damage and UVR-induced cytokine expression will in turn trigger strong p38 MAPK activation. p38 MAPK activation is required for normal p53 activation and consequent apoptosis signaling (Ziegler et al., 1994; Hildesheim et al., 2002). The additive effects of apoptosis and inflammation will culminate in epidermal erosion and loss of its barrier function, which in turn may stimulate keratinocyte hyperproliferation to regenerate the damaged epidermis. This regenerative/hyperproliferative response probably also involves p38 signaling through AP-1 activation (Chen and Bowden, 2000). Although JNK and ERK MAPK are also activated by UVR (Kyriakis and Avruch, 1996; Rosette and...
Karin, 1996; Ip and Davis, 1998), our results clearly indicate that p38 MAPK is a key factor involved in mediating the detrimental effects of acute UVR. We have demonstrated that treating mice with the p38 inhibitor SB202190 is protective against downstream effects of UVR stress-induced MAPK activation, namely, apoptosis, inflammation, and reactive hyperproliferation, which are manifested as eosinophilic sunburn cells, subcorneal pustules, and epidermal thickening, respectively.

Our results indicate that p38 inhibitors may have therapeutic value in the treatment of individuals exposed to cytotoxic doses of DNA-damaging agents. For example, many important types of stress in addition to UVR, such as alkylating agents, oxidizing agents, osmotic shock, and others (Garmyn et al., 2001; Wilhelm et al., 1997; Pandey et al., 1999; Suh et al., 2000; Suh and Park, 2001; Aggeli et al., 2002; Cheng et al., 2002), trigger a rapid and robust p38 MAPK response, whereas ionizing radiation typically does not in most cell types (Liu et al., 1996). Based on our results, treating mice with the p38 inhibitor SB202190 is protective against downstream effects of UVR stress-induced MAPK activation, namely, apoptosis, inflammation, and reactive hyperproliferation, which are manifested as eosinophilic sunburn cells, subcorneal pustules, and epidermal thickening, respectively.

Figure 3
Effects of SB202190 on UVR-induced p38 and JNK MAPK activation. Immunohistochemical staining was performed on whole skin of control mice or UV-irradiated and/or SB202190-treated mice for (A) phosphorylated p38 (p-p38), (B) phosphorylated JNK (p-JNK), (C) p21, and (D) phosphorylated c-Jun (p-c-Jun). (A) Basal keratinocyte staining (arrowheads) for p-p38 is evident in UV-irradiated skin with (bottom right panel) or without (bottom left panel) SB202190 treatment. No p-p38 staining is detected in unirradiated control tissues, irrespective of SB202190 treatment (top panels). (B) p-JNK epidermal staining of both basal and suprabasal keratinocytes (arrowheads) is also detected in UV-irradiated skins independent of the inhibitor (bottom panels) but absent in unirradiated controls (top panels). (C) Nuclear p21 staining of UV-irradiated skin is only present in the basal keratinocytes of untreated UV-irradiated skin in accordance with p-p38 localization (bottom left panel). SB202190-treated mice have virtually no p21 accumulation after UVR (bottom right panel). (D) c-Jun is readily phosphorylated by UVR-activated JNK independently of SB202190 and is expressed in all epidermal layers, consistent with p-JNK (bottom panels). Images are representative of three animals per group. Scale bar, 20 μm.

Figure 4
SB202190 effect on acute UVR-induced inflammation and hyperproliferation. Hematoxylin and eosin staining of formalin-fixed skin shows several features associated with UV irradiation, such as the presence of sunburn/apoptotic cells with dyskeratotic and vacuolated cytoplasm and pyknotic nuclei (arrows, panel iii); pronounced epidermal thickening resulting from proliferation (panel iii); hyperkeratosis due to excessive keratin expression (panel iii); and a strong inflammatory response manifested by the emergence of subcorneal pustules (panel v). These detrimental effects are dramatically reduced with SB202190 treatment (panels ii and iv). Representative images are shown for experiments performed on three animals. Scale bar, 20 μm.
treatment with p38 inhibitors, such as SB202190, would be expected to reduce acute injury responses to a variety of damaging agents including blistering agents such as alkylation agents. Acute morbidity could be reduced if the barrier function of damaged skin was maintained by reducing apoptosis and consequent inflammatory responses.

Contrary to its beneficial effects against acute skin injuries, the detrimental effects of long-term use of p38 inhibitors also need to be considered. The use of these compounds in the treatment of chronic inflammatory conditions or even chronic/repeated sun damage, for instance, may not only compromise the ability of tissues to monitor the genomic integrity of their cells but also their ability to eliminate potentially tumorigenic cells (Hildesheim et al, 2002). Chronic administration of p38 inhibitors may well have utility in treatment of inflammatory conditions (Barnes, 2001; Schultz, 2003), but such patients probably need to reduce exposure to genotoxic agents, such as UVR and other sources such as cigarette smoke. Chronic exposure to solar radiation, for instance, is known to induce mutations in the Tp53 gene. In epidermal keratinocytes, Tp53 mutations are frequently present in precancerous lesions and are considered an early event in the progression to nonmelanoma skin cancers (Ling et al, 2001). In this scenario, SB202190 treatment may in fact impede the patients’ ability to eliminate Tp53 mutant epidermal keratinocyte by reducing removal of damaged cells via apoptosis. Other mutations leading to disruption of tumor suppressors and/or activation of oncogenes may also arise in severely damaged cells, which escape the normal p38–p53 surveillance mechanism. Therefore, use of p38 inhibitors should not be considered “sunscreen equivalents” and careful monitoring for increased carcinogenic risk may also be warranted in such patients.

Materials and Methods

Mouse sunburn and terminal deoxynucleotidyl transferase dUTP nicked end-labeling (TUNEL) assay Adult male C57BL6/129 mice 6 wk of age were subject to UBV irradiation with Westinghouse FS20 sunlamp bulbs according to published procedures (Hildesheim et al, 2002). Briefly, animals were anesthetized with avertin (2.5%), shaved, and irradiated while under sedation with 2000 Jm–2 UBV. Animals treated with the SB202190 p38 inhibitor (Calbiochem) were subject to three intraperitoneal injections of 8 mg SB202190 per kilogram of body weight at 8-h intervals. The first injection was performed 30 min intraperitoneal injections of 8 mg SB202190 per kilogram of body weight at 8-h intervals. The first injection was performed 30 min

Immunohistochemistry Five-micrometer-thick sections of formalin-fixed and paraffin-embedded tissues were subject to standard immunohistochemistry as specified by the manufacturer (Cell Signaling Technology). Sections were first deparaffinized in xylene and then rehydrated by sequentially washing them in 100% ethanol, 95% ethanol, water, and phosphate-buffered saline. Antigen retrieval was then performed with 10 mM sodium citrate, pH 6.0, followed by blocking with either 5% goat serum (for polyclonal antibodies) or 5% horse serum (for monoclonal antibody) in phosphate-buffered saline. Next, specimens were incubated with either anti-p-p38 (1:50), p-SAPK/c-jun N-terminal kinase (JNK) (1:100), p-c-Jun (1:50) monoclonal antibodies from Cell Signaling Technology or with anti-p21 (F-5, 1:100) monoclonal antibody from Santa Cruz Biotechnology. Detection of primary antibodies was performed with the peroxidase ABC kit as per manufacturer’s specifications (Vector Laboratories).

DOI: 10.1046/j.0022-202X.2004.22229.x

Manuscript received July 2, 2003; revised September 30, 2003; accepted for publication October 14, 2003

References


References


p38 INHIBITOR PROTECTS AGAINST UV IRRADIATION

501


Accepted for publication October 14, 2003.