Role of p38 MAPK in UVB-Induced Inflammatory Responses in the Skin of SKH-1 Hairless Mice

Arianna L. Kim,* Jeffrey M. Labasi,† Yucui Zhu,* Xiuwei Tang,* Kim McClure,† Christopher A. Gabel,† Mohammad Athar,* and David R. Bickers*

*Department of Dermatology, Columbia University College of Physicians & Surgeons, New York, New York, USA; †Department of Inflammation, Pfizer Inc. Global Research & Development, Groton, Connecticut, USA

The p38 mitogen-activated protein kinase (MAPK) signaling pathway is activated by numerous inflammatory mediators and environmental stresses. We assessed the effects of ultraviolet B (UVB) on the p38 MAPK pathway and determined whether cyclooxygenase (COX)-2 expression is downstream of this kinase in the skin of UVB-irradiated SKH-1 mice. SKH-1 mice were irradiated with a single dose of UVB (360 mJ per cm^2), and activation of the epidermal p38 MAPK pathway was assessed. UVB-induced phosphorylation of p38 MAPK occurred in a time-dependent manner. Phosphorylation of MAPK-activated protein kinase-2 (MAPKAPK-2) also was detected and correlated with an increase in its kinase activity. Phosphorylation of heat shock protein 27 (HSP27), a substrate for MAPKAPK-2, also was detected post-irradiation. Oral administration of the p38 inhibitor, SB242235, prior to UVB irradiation, blocked activation of the p38 MAPK cascade, and abolished MAPKAPK-2 kinase activity and phosphorylation of HSP27. Moreover, SB242235 inhibited expression of the pro-inflammatory cytokines interleukin (IL)-6 and KC (murine IL-8) and COX-2. Our data demonstrate that UVB irradiation of murine skin activates epidermal p38 MAPK signaling and induces a local pro-inflammatory response. Blockade of the p38 MAPK pathway may offer an effective approach to reducing or preventing skin damage resulting from acute solar radiation.

Key words: COX-2/inflammatory response/mice/p38 MAPK/UVB
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p38 mitogen-activated protein kinase (MAPK) belong to a highly conserved family of serine/threonine protein kinases that include extracellular signal-regulated protein kinases (ERK) and c-Jun N-terminal kinases (JNK). p38 MAPK is involved in numerous cellular processes, such as cell growth and survival, differentiation, development, cell cycle regulation, and cell death, depending on the cell type and stimulus (Juretic *et al*, 2001; Yosimichi *et al*, 2001). The ERK pathway is generally activated by mitogenic stimuli, whereas the JNK and p38 pathways are activated by pro-inflammatory or stressful stimuli. Various stressors, such as ultraviolet (UV) radiation, oxidative injury, heat shock, cytokines, and other pro-inflammatory stimuli, are known to trigger induction of the p38 MAPK-dependent signaling cascade. p38 MAPK exists as four isoforms (α, β, γ, and δ) and is activated by several upstream kinases *in vitro*, including MAP kinase kinases (MKK) 3, 4, and 6 (Kyriakis and Avruch, 2001). Phosphorylation of p38 MAPK in turn activates numerous downstream substrates, including p90 MAPK-activated protein kinase-2 (MAPKAPK-2) (Maizels *et al*, 2001) and mitogen and stress-activated kinase-1/2 (MSK1/2) (Deak *et al*, 1998). p90 MAPKAPK-2 and MSK1/2 function to phosphorylate heat shock protein 27 (HSP27) and cAMP-response element binding protein transcriptional factor, respectively (Kato *et al*, 2001; Wiggin *et al*, 2002). Other transcription factors, including activating transcription factor 2, Elk, CHOP/GADD153, and myocyte enhancer factor 2, are known to be regulated by these kinases (Shi and Gaestel, 2002).

Exposure of skin to both acute and chronic solar UVB radiation is the major known environmental agent driving the development of basal cell carcinomas and squamous cell carcinomas (SCC), also known as non-melanoma skin cancers. These are the most common types of human malignancy affecting more than one million Americans each year. In addition to its direct mutagenic effects, UVB induces inflammatory responses that are critical for tumor induction (Wilgus *et al*, 2002). The UVB-induced inflammatory response is characterized by the acute development of edema and erythema, and increases in dermal inflammatory cell infiltrates and augmented prostaglandin synthesis (Hruza and Pentland, 1993; Terui and Tagami, 2000).

Cyclooxygenases (COX) convert free arachidonic acid into a series of pro-inflammatory eicosanoids including prostaglandins. The COX-1 isoform serves as a housekeeping enzyme and is expressed in most tissues, whereas

Abbreviations: ATF2, activating transcription factor 2; COX-2, cyclooxygenase 2; ERK, extracellular signal-regulated kinase; HSP27, heat shock protein 27; IL, interleukin; JNK, c-Jun N-terminal kinase; KC, mouse KC/N51 originally identified as a PDGF (platelet-derived growth factor)-induced immediate early gene, known as the functional homolog of IL (interleukin)-8; MAPK, mitogen-activated protein kinase; MAPKAPK-2, MAPK-activated protein kinase-2; MKK, MAP kinase kinase; MSK1/2, mitogen and stress-activated kinase-1/2; NFκB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; UVB, ultraviolet B

COX-2 is an inducible isoform generated in response to pro-inflammatory stimuli, including cytokines. The role of p38 MAPK in UVB-induced COX-2 expression has been documented by several laboratories using a human keratinocyte cell line, HaCaT. In this cell system, p38 MAPK, but not ERK, was required for UVB-induced COX-2 expression (Chen *et al*, 2001). In contrast, Ashida *et al* (2003) recently reported that UVB induction of COX-2 is associated with the activation of EGFR, ERK, p38 MAPK, and PI (phosphatidylinositol) 3-kinase. p38 MAPK transcriptionally regulates COX-2 in HaCat cells following UVB irradiation (Chen *et al*, 2001; Tang *et al*, 2001). The involvement of p38 MAPK in regulation of the stability of the COX-2 message was also demonstrated in interleukin (IL)-1-treated HeLa cells and lipopolysaccharide-treated primary human monocytes (Ridley *et al*, 1998; Dean *et al*, 1999; Lasa *et al*, 2000). These data suggest that UVB-induced p38 MAPK activity and its attendant pro-inflammatory signaling are involved in the pathogenesis of skin disorders including SCC.

In this study, we have assessed the *in vivo* effects of UVB irradiation on acute inflammatory responses in SKH-1 hairless mice, a well-established model for UVB-induced skin carcinogenesis. Our data indicate that acute UVB exposure induces the rapid activation of the p38 kinase signaling cascade, leading to COX-2 and pro-inflammatory cytokine production. Furthermore, oral administration of a p38 MAPK inhibitor abrogated these UVB-mediated inflammatory responses.

Results

UVB activates p38 MAP kinase signaling in murine skin Irradiation of SKH-1 mice with a single dose of 360 mJ per cm^2 of UVB-induced consistent visible erythema and edema, and this dose was used throughout this study. To demonstrate that UVB-induced activation of p38 MAPK signaling *in vivo*, mice were irradiated and sacrificed at various time points thereafter (0.5, 2, 8, and 24 h). Dorsal skin patches were excised and the dermis was removed by scraping, and extracts of the epidermal samples were prepared as described in Materials and Methods. Rapid phosphorylation of p38 MAPK was documented following analysis of these extracts by western blotting using a phospho-specific antibody (Fig 1). p38 MAPK phosphorylation was detected as early as 30 min post-irradiation, and was maximal at 24 h. No phosphorylation was detected in non-irradiated skin. Phosphorylation of MAPKAPK-2, a downstream substrate of p38 MAPK, was detected 30 min post-irradiation and gradually increased to its maximum level by 24 h. The total endogenous p38 MAPK and MAPKAPK-2 levels were not altered by UVB irradiation (Fig 1). Western blot analysis also demonstrated that kinases upstream of p38 (MKK3/6) were phosphorylated immediately following UVB irradiation (data not shown).

UVB induces phosphorylation of MAPKAPK-2 at Thr 222 and Thr 334 MAPKAPK-2 activation by p38 MAPK requires the phosphorylation of two threonine sites at positions 222 and 334. We assessed the phosphorylation pattern of these two residues after UVB irradiation. Skin sections from UVB-irradiated and non-irradiated mice were subjected to immunohistochemical staining using Thr222- and Thr334-phospho-specific MAPKAPK-2 antibodies. Phosphorylated MAPKAP-2 proteins in both residues were detectable throughout the epidermis following UVB irradiation, and 24 h later, all epidermal cells stained positively for the phospho-MAPKAPK-2 protein (Fig 2). The staining was minimal in the non-irradiated control skin. To distinguish the basal layer staining, the skin section (1 h post-UVB) was stained for proliferating cell nuclear antigen (PCNA), which is an established marker of cellular proliferation in both normal and neoplastic tissues and is required for DNA replication. PCNA expression was noted to be more intense in the basal layer (Fig 2*f*).

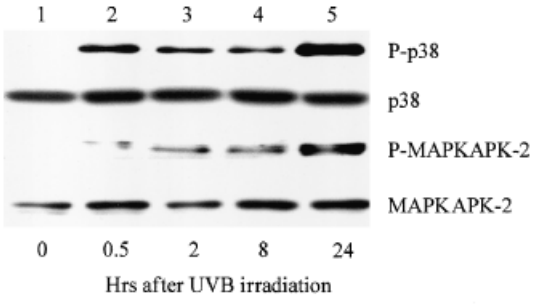

Figure 1
p38 mitogen-activated protein kinase (MAPK) pathway is activated by ultraviolet B (UVB) irradiation of murine skin *in vivo*. Total and phosphorylated levels of p38 MAPK and MAPK-activated protein kinase-2 (MAPKAPK-2) were detected by western blotting of epidermal extracts prepared from non-irradiated control (*lane 1*) and UVB-irradiated skin (*lanes 2–5*). Mice were sacrificed at 30 min (*lane 2*), 2 h (*lane 3*), 8 h (*lane 4*), and 24 h (*lane 5*) after UVB irradiation. Each lane contains 80 μg of protein lysate.

UVB-induced MAPKAP kinase-2 activity correlates with HSP27 phosphorylation MAPKAPK-2 kinase activity was assessed in UVB-irradiated skin extracts by immunoprecipitating with an anti-MAPKAPK-2 antibody and assaying these complexes for kinase activity, using an MAPKAPK-2 substrate peptide; incorporation of ^{32}P-ATP into the substrate peptide was measured by scintillation counting. Figure 3*B* compares MAPKAP kinase-2 activity in the non-irradiated control skin and the UVB-irradiated skin at 1, 3, and 24 h post-irradiation. Compared with the non-irradiated control, a 5-fold ($p = 0.018$) increase in kinase activity was observed at 3 h, and increases of 2.5-fold ($p = 0.032$) and 4.4-fold ($p = 0.003$) were observed at 1 and 24 h, respectively. Moreover, activation of MAPKAPK-2 correlated with the appearance of phospho-HSP27 substrate (Fig 3*A*). These data indicate that a single skin exposure to UVB activates the p38 MAPK signaling cascade in the skin of SKH-1 hairless mice. This finding is consistent with studies demonstrating that p38 kinase is involved in responses to UVB in murine and human cells *in vitro* (Bulavin *et al*, 2001).

The p38 MAPK inhibitor SB242235 abolishes MAPKAPK-2 activity and HSP27 phosphorylation To further explore the role of p38 in mediating UVB-induced inflammatory responses, we administered the p38 MAPK inhibitor

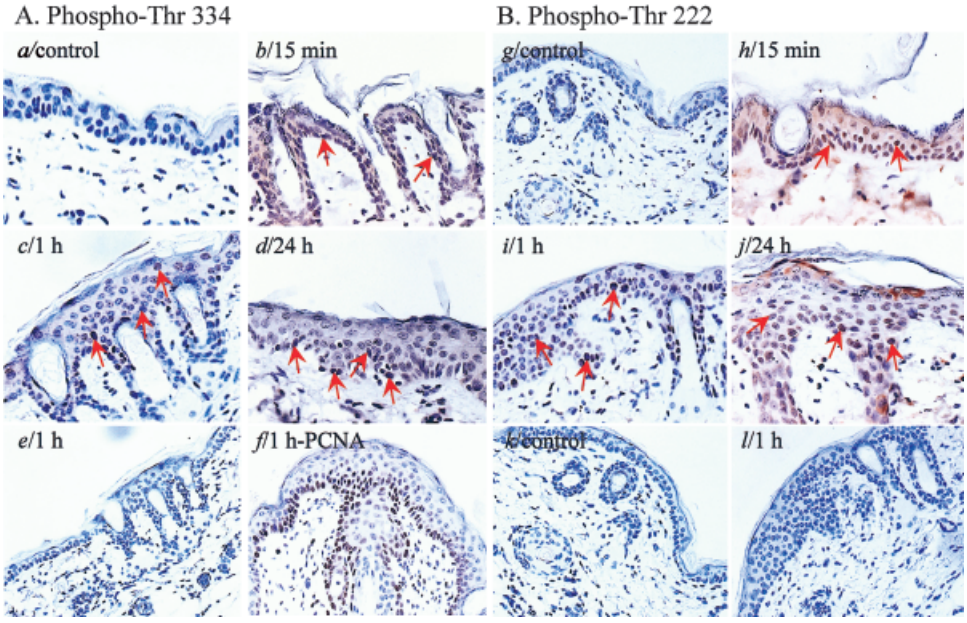

Figure 2
Ultraviolet B (UVB) induces phosphorylation of mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK-2) in murine epidermis. Immunohistochemical detection of phospho-MAPKAPK-2 in UVB-irradiated murine skin sections, using phospho-specific antibodies for Thr334 (*A*) and Thr222 (*B*), in non-irradiated control skin (*a*, *g*) and skin prepared 15 min (*b*, *h*), 1 h (*c*, *i*), and 24 h (*d*, *j*) after UVB irradiation (360 mJ per cm^2). (*e*, *l*), which were adjacent sections of (*c*) and (*i*), respectively, stained omitting anti-phospho-specific antibodies for Thr334 and Thr222. (*k*) A non-irradiated murine skin section, stained omitting anti-phospho-specific antibodies for Thr222. (*f*) One hour post-irradiation, stained with anti-proliferating cell nuclear antigen (PCNA) antibody. The arrows indicate the cells that stained positively for phospho-MAPKAPK-2. (magnification: × 200; *Scale bar* = 100 μm).

SB242235 (Badger *et al*, 2000a, b) orally to mice 30 min prior to UVB irradiation. Epidermal extracts from these mice were analyzed for HSP27 phosphorylation by western blotting (Fig 4*A*) and for MAPKAPK-2 kinase activity (Fig 4*B*). As shown in Fig 4*A*, HSP27 phosphorylation induced by UVB was abolished at 1 h (*lanes 3–6*) and significantly reduced at 3 h (*lanes 7–10*) and 24 h (*lanes 11–14*) in mice treated with SB242235, relative to vehicle-treated controls. The level of total endogenous HSP27 is not altered by UVB irradiation (Fig 4*A*). SB242235 treatment completely abolished MAPKAPK-2 kinase activity at the earlier time points (1 and 3 h), which was returned to baseline at 24 h (Fig 4*B*). Figure 4*C* compares the level of MAPKAPK-2 kinase activity observed in the skin of SB242235-treated animals (dotted line) with plasma concentration of SB242235 (solid line) at 1, 3, and 24 h post-UVB irradiation. Plasma concentrations of SB242235 (4.5 μg per mL) were maximal at 1 h post-dose and gradually decreased; at 24 h, no plasma-associated drug was detectable.

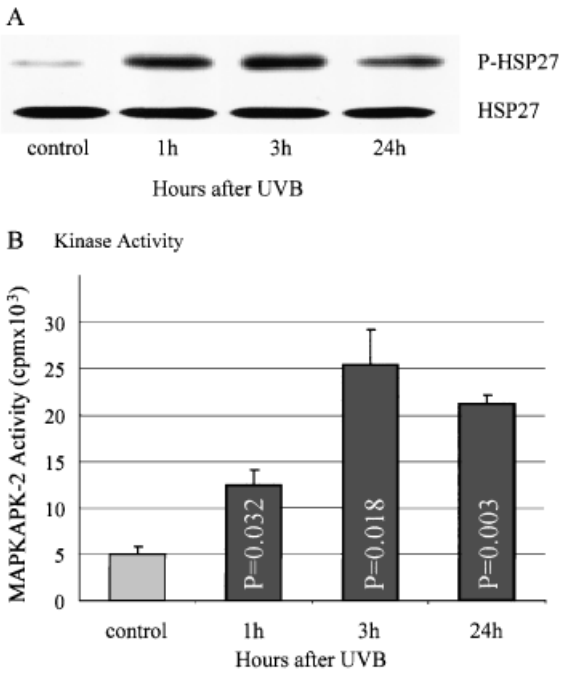

Figure 3
Ultraviolet B (UVB)-induced mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK-2) kinase activity correlates with heat shock protein 27 (HSP27) phosphorylation. (*A*) The presence of phosphorylated HSP27 in epidermal extracts was detected by western blotting using a phospho-specific HSP27 antibody. Total HSP27 is shown as a control. Each lane contains 80 μg of protein. (*B*) MAPKAPK-2-associated kinase activity was measured in UVB-irradiated skin extracts. MAPKAPK-2-dependent protein kinase was immunoprecipitated from 250 μg of protein lysate, and its kinase activity was assayed using substrate peptide and γ-^{32}P-ATP; incorporation of ^{32}P into the peptide was measured by scintillation counting. Kinase activity of the non-irradiated control was compared with samples taken at 1, 3, and 24 h following UVB irradiation. Each point represents the mean of four animals. p-values, compared with non-irradiated control.

p38 inhibition blocks UVB-induced COX-2 expression We have previously shown that COX-2 expression is enhanced by UVB irradiation and that COX-2 is overexpressed in UVB-induced SCC in SKH-1 mice (Athar *et al*, 2001; An *et al*, 2002). We therefore determined whether p38 MAPK could be involved in UVB-induced COX-2 expression. Mice were irradiated with UVB and COX-2 protein detected by western blotting of epidermal extracts. COX-2 protein was detectable 3 h following UVB irradiation (*lanes 7–10*) and increased further by 24 h (*lanes 11–14*) (Fig 5*A*). Non-irradiated epidermal samples (two mice: *lanes 1* and *2*) and the irradiated epidermis at 1 h following UVB (*lanes 3–6*) showed no detectable COX-2 protein. SB242235 administration significantly reduced the level of COX-2 protein observed 3 h after UVB irradiation. COX-2 expression, however, was observed in some animals at the 24 h time point. Since COX-2-derived metabolites are components of inflammatory responses, we asked whether the p38 MAPK inhibitor influenced the development of UVB erythema. UVB-induced erythema in these mice generally peaks 5 d after exposure. Figure 5*B* shows a representative picture of animals exposed to 360 mJ per cm^2 on day 5 (*left panel*: *a*).

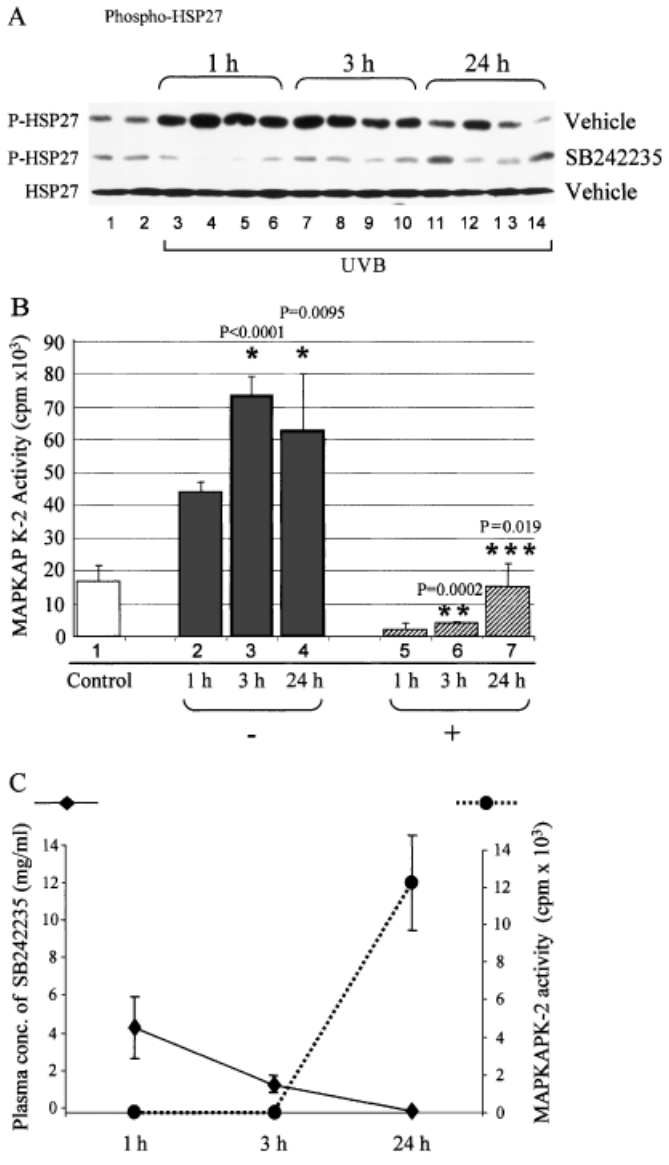

Figure 4
Oral administration of SB242235 inhibits mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK-2) activity and heat shock protein 27 (HSP27) phosphorylation in skin *in vivo*. A total of 24 SKH-1 hairless mice were divided into two groups. One group of 12 animals received orally administered SB242235 30 min prior to ultraviolet B (UVB) irradiation (360 mJ per cm^2). Controls received vehicle only. Four mice from each group were sacrificed at 1, 3, and 24 h following irradiation. (*A*) The effect of SB242235 on UVB-induced HSP27 phosphorylation was assayed by western blotting. *Lanes 1–2*: non-irradiated control epidermal extracts and *lanes 3–14*: epidermal extracts from UVB-irradiated skin. (*B*) Effects of SB242235 on UVB-induced MAPKAPK-2 kinase activity. Epidermal extracts from four mice for each time point were assayed for MAPKAPK-2 kinase activity. Kinase activity recovered in skin from mice treated with (+) or without (−) SB242235 was compared with that associated with non-irradiated control skin samples. Each point represents the mean of four animals. *Compared with lane 1; ** and ***compared with lanes 3 and 4, respectively. (*C*) Plasma concentration of SB242235. The solid line indicates SB242235 plasma concentration at 1, 3, and 24 h following UVB irradiation. The dotted line indicates the MAPKAPK-2 kinase activities measured in skin samples from these same animals. Each point represents the mean of four animals.

Variable erythema was seen in these four mice, likely reflecting the fact that the animals are free to move around the cage during irradiation. Typically, four mice in a cage are irradiated simultaneously. Although the degree of redness varied among the four animals, each showed clear evidence of erythema. A single oral dose of SB242235 administered 30 min prior to UVB irradiation virtually eliminated the development of erythema in these mice (*right panel*: *b*).

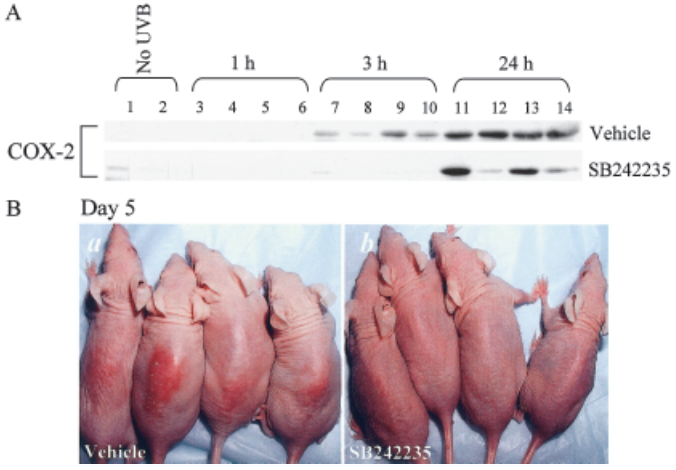

Figure 5
Inhibition of ultraviolet B (UVB)-induced cyclooxygenase (COX)-2 expression and UVB erythema by SB242235. (*A*) COX-2 protein was determined by western blotting using anti-COX-2 antibody, 80 μg of protein per lane. (*B*) SKH-1 mice were treated with either SB242235 or the vehicle 30 min prior to UVB irradiation (360 mJ per cm^2). The mice were inspected daily basis for the appearance of erythema on UVB-irradiated dorsal skin. Pictures were taken on day 5 following irradiation. The left panel (*a*) shows vehicle-treated controls, and the right panel (*b*) shows animals treated with SB242235.

UVB-induced pro-inflammatory cytokine production is blocked by the p38 inhibitor Keratinocytes are major contributors to epidermal cytokine production either constitutively or in response to various stimuli involved in the inflammatory responses, as well as cell proliferation and differentiation processes (Grone, 2002). IL-6 and IL-8 are among the best-characterized keratinocyte-derived pro-inflammatory cytokines (Ansel *et al*, 1990; Luger *et al*, 1990; McKenzie *et al*, 1994). IL-6 is induced in keratinocytes exposed to UVB radiation or treated with TGF-α (Aragane *et al*, 1996; Pupe *et al*, 2002), and is reported to drive keratinocyte proliferation associated with epidermal hyperplasia (Grossman *et al*, 1989; Turksen *et al*, 1992; Sawamura *et al*, 1998). IL-8 is a potent neutrophil attractant and activator. The mouse homolog of IL-8 is known as KC, the production of which is increased in various inflammatory conditions (Farone *et al*, 1995; Godiska *et al*, 1995). Its activity is mediated by the murine IL-8 receptor. We assessed the production of IL-6 and KC in UVB-irradiated murine epidermis and determined whether p38 MAPK was involved in their production. The levels of both KC and IL-6 increased substantially in response to UVB, showing a 9-fold ($p = 0.0007$) and 3-fold ($p = 0.001$) induction, respectively, at 24 h after irradiation, as compared with the non-irradiated control (Fig 6). A significant reduction in the levels of KC and IL-6 was observed with SB242235 treatment. These results demonstrate that the p38 MAP kinase signaling pathway is necessary for UVB-induced cytokine expression in murine skin.

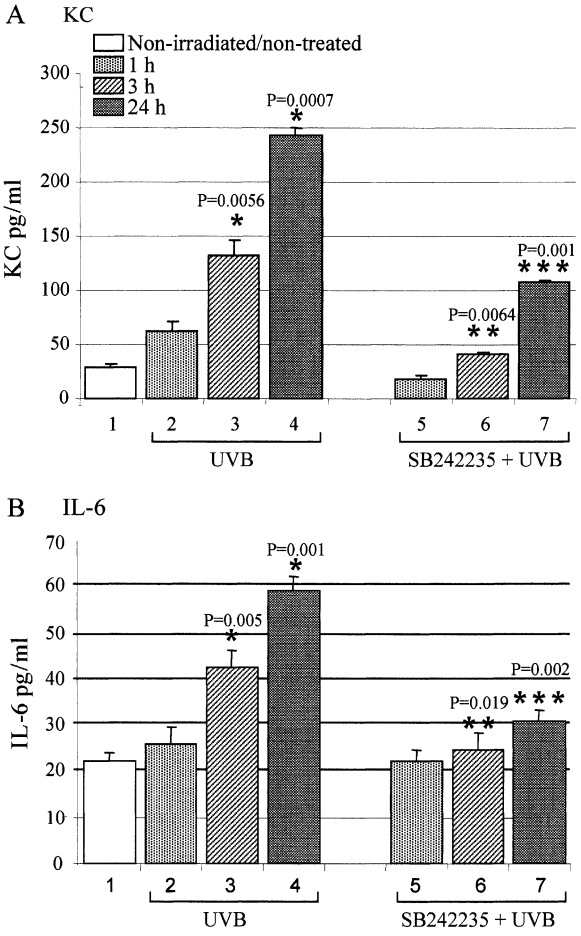

Figure 6
Inhibition of p38 mitogen-activated protein kinase signaling leads to reduced interleukin (IL)-6 and KC (IL-8) production. Inhibition of KC (*A*) and IL-6 (*B*) production by SB242235 administration in epidermal extracts, measured at 1, 3, and 24 h after UVB irradiation. *p-value compared with non-irradiated control (*lane 1*); **compared with 3 h post-irradiation (*lane 3*); ***compared with 24 h post-irradiation (*land 4*). The KC and IL-6 data were derived from a total of four mice for each time point. Each data point represents the mean + SD.

Discussion

Sunburn is a visible cutaneous manifestation of activation of inflammatory signaling pathways resulting from environmental exposure to solar radiation, particularly UVB. Furthermore, sun exposure is clearly the most important factor in the development of skin cancer. In this study, we used SKH-1 hairless mice to determine the role of the p38 MAPK signaling pathway in the inflammatory response to UVB. Our results demonstrate that UVB activates the p38 MAPK pathway, inducing acute inflammatory responses and erythema. The time-dependent activation of p38 MAPK signaling correlates with UVB-mediated elaboration of the pro-inflammatory cytokines IL-6 and murine KC (IL-8), as well as COX-2. p38 MAPK pathway activation was confirmed by detecting phosphorylation of MAPKAPK-2 and HSP27 and by directly measuring the kinase activity of MAPKAPK-2. Phosphorylation of MAPKAPK-2 occurred rapidly (within 15 min) after UVB exposure, suggesting that p38 MAPK signaling is involved in the immediate response to this form of energy. Acute responses to UVB are driven by inflammatory pathways and may reflect the direct effects of absorbed photons on DNA (Tedesco *et al*, 1997), through the involvement of UVB-generated reactive oxygen species (Athar *et al*, 1989; Dalle Carbonare and Pathak, 1992), or the generation of prostaglandins and other cytokines (Pentland and Needleman, 1986; Kupper, 1990; Soter, 1990).

Synthesis of many cytokines, such as tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-10, and IL-8, is induced by UVB in keratinocytes (Konnikov *et al*, 1989; Kirnbauer *et al*, 1991; Grandjean-Laquerriere *et al*, 2002), and these drive cutaneous inflammatory responses (Kondo, 1999). p38 MAPK is an important component of cytokine signaling pathways (Allen *et al*, 2000). In A431 cells, for example, p38 MAPK mediates IL-6 synthesis in response to IL-4, and inhibition of p38 MAPK reduces the stability of IL-6 mRNA (Wery-Zennaro *et al*, 2000). Our results show that IL-6 and KC (IL-8) are induced in SKH-1 murine skin following UVB irradiation. Furthermore, oral administration of the p38 MAPK inhibitor SB242235 completely inhibited the production of these cytokines as well as COX-2 expression, suggesting that regulation of COX-2 is dependent on p38 MAPK. Recently, MAPKAPK-2 was shown to contribute to increased COX-2 mRNA stability following taxane treatment, and a selective p38 MAPK inhibitor suppressed taxane-mediated COX-2 stimulation in human mammary epithelial cells (Subbaramaiah *et al*, 2003).

Following oral administration of SB242235, the UVB-induced inflammatory response was attenuated. This agent effectively suppressed activation of MAPKAPK-2 and phosphorylation of HSP27 following UVB irradiation. SB242235 also suppressed UVB-induced induction of COX-2 and the cytokines IL-6 and KC. These pharmacological effects were manifested within 1 and 3 h of irradiation, but returned to baseline by 24 h. SB242235 demonstrated a short plasma half-life, and at the dose used in this study, plasma levels of the compound were undetectable after 24 h, suggesting that its disappearance coincided with loss of its pharmacological effects. Moreover, a single oral dose of SB242235 effectively abrogated erythema development in UVB-irradiated murine skin. The spectral property of SB242235 showed that it absorbs UVR between 290 and 380 nm, with maximum absorption at 330 nm. In order to determine whether the oral administration of SB242235 exerts a photoprotective effect in the epidermis, we measured thymine dimers in epidermal DNA, which is a direct index for DNA damage following UV radiation of the skin (Antille *et al*, 2003). We found that UVB irradiation induces the formation of thymine dimers throughout the epidermis, as demonstrated by immunohistochemical staining. The number and density of epidermal nuclei stained for thymine dimers, however, were not affected by pre-treatment with SB242235, indicating that intracellular SB242235 is not functioning as a sunscreen to protect against UVB-induced DNA damage, and that the SB242235-mediated anti-inflammatory response we observed is likely because of inhibition of the p38 MAPK pathway (data not shown). Taken together, our data suggest that pharmacological suppression of the acute biochemical changes by SB242235 is sufficient for attenuation of the entire UVB-induced inflammatory responses.

Inflammatory cytokines such as TNF-α and IL-1 are known to regulate IL-6 production (Eliopoulos *et al*, 1999). TNF-α-mediated IL-6 induction occurs through stimulation of nuclear factor-κB (NF-κB), in which p38 MAPK acts as an activator of IB kinases (Craig *et al*, 2000). In our study, trace amounts of TNF-α were detected in response to UVB, using both western blotting and ELISA (data not shown). It is known that TNF-α and IL-6 can be differentially regulated in UVB-irradiated human keratinocytes via the cyclic AMP/protein kinase A pathway (Grandjean-Laquerriere *et al*, 2003). IL-6 is overexpressed in some human tumors where its synthesis is thought to be regulated by the coordinated activation of the NF-κB and p38 pathways. Inhibition of NF-κB and p38 MAPK activation can suppress IL-6 synthesis in prostate cancer cells (Park *et al*, 2003). Furthermore, potential binding sites for NF-κB, in addition to binding sites for other transcription factors, are present in the IL-6 promoter (Asschert *et al*, 1999; Franchimont *et al*, 1999). UVB is known to activate NF-κB in both keratinocytes and whole-skin extracts, linking the p38 MAPK and NF-κB pathways in response to UVB. The elucidation of the mechanism by which chronic UVB irradiation induces cytokine synthesis in the skin, and its link to the p38 MAPK signal transduction pathways and NF-κB may provide important insights into the pathogenesis of UVB-induced skin disorders.

Materials and Methods

The medical ethical committee of the University of Columbia approved all described studies.

Animals Female SKH-1 hairless mice (4–6 wk of age) were purchased from Charles River Laboratories (Kingston, New York). All experiments were performed in accordance with the Columbia University Animal Care guideline and protocols. The SKH-1 hairless mice strain was chosen because these animals can be easily irradiated and observed for UVB erythema and tumor induction effects. In contrast to many other immunodeficient hairless mice strains, such as BALB/c (nu/nu), SKH-1 mice are immunocompetent.

UV light source and irradiation The UV Irradiation Unit (Daavlin, Bryan, Ohio) used in this study is described in our previous publication (Kim *et al*, 2002). For irradiation, the SKH-1 hairless mice were exposed to 360 mJ per cm^2 of UVB with four mice per cage.

***In vivo* inhibition of p38 MAPK** Mice were fasted overnight with free access to water. The p38 MAPK inhibitor, SB242235, was administered by oral gavage, 100 mg per kg body weight in 0.5% methylcellulose (Sigma-Aldrich, St Louis, Missouri), 30 min prior to UVB exposure. Control mice were treated with 0.5% methylcellulose suspension alone. All mice were returned to their normal diet following UVB irradiation. SB242235 was prepared as previously described (Adams *et al*, 1998, 2001; Ward *et al*, 2002).

Immunohistochemical analyses Immunohistochemical staining was performed as previously described (Kim *et al*, 2002) using the following primary antibodies (1:500 dilutions of anti-phospho-MAPKAPK-2 (Thr334) and anti-phospho-MAPKAPK-2 (Thr222), Cell Signaling Tech. (Beverly, Massachusetts); anti-PCNA antibody, Calbiochem (LaJolla, California)).

Western blot Skin specimens from both the UVB-irradiated mice treated with (+) or without (−) the p38 inhibitor, and the non-irradiated mice were excised, and the dermis was removed by scalpel scraping. These epidermal samples were homogenized in a lysis buffer and western blot analyses were performed as previously described (Kim *et al*, 2002). The following antibodies were used in this study: anti-p38 and anti-HSP27, Santa Cruz Biotech. (Santa Cruz, California); anti-phospho-MAPKAPK-2 (Thr334), anti-MAPKAPK-2, anti-phospho-p38 (Thr180/Tyr182), and anti-phospho-HSP27 (Ser82), Cell Signaling Tech.; and anti-COX-2, Cayman Chemicals (Ann Arbor, Michigan). Antibody-reactive proteins were detected using ECL reagents according to the manufacturer's instructions (Amersham, Piscataway, New Jersey), with several exposures made on Kodak X-Omat X-ray films.

MAPKAP kinase 2 assay Lysates were pre-cleared with 300 μL of 10% protein G sepharose slurry (Sigma-Aldrich) for 10 min at 4°C, after which supernatants were collected and protein concentration was determined by DC Bio-Rad assay (Hercules, California). Samples containing 500 μg of protein were then incubated with 50 μg sheep anti-rabbit MAPKAP kinase 2 antibody (Upstate Biotech., Waltham, Massachusetts) in 500 μL buffer A (50 mM Tris, pH 7.5, 1% Triton X-100, 50 mM sodium fluoride, 10 mM β-glycerol phosphate, 1 mM EDTA, 1 mM EGTA, 0.5 mM sodium orthovanadate, 1 mM DTT, 0.1 mM PMSF, 10 nM okadaic acid, 5 mM sodium pyrophosphate, and complete EDTA-free protease inhibitor cocktail (Roche Applied Science, Indianapolis, Indiana) for 2 h at 4°C. Three hundred microliters of 10% protein G sepharose slurry was then introduced and the mixture was incubated for 1 h at 4°C. The beads were collected by centrifugation at 3000 r.p.m. (× 0.8 *g*) for 3 min at 4°C. Immune complexes were washed once in 0.5 mL of buffer A, containing 0.5 M NaCl; once in 1 mL of buffer A; and once in 0.5 mL of ADB buffer (20 mM MOPS, pH7.2; 25 mM β-glycerol phosphate; 5 mM EGTA; 1 mM sodium orthovanadate; and 1 mM DTT). Finally, the bead pellets were suspended in 40 μL of ADB buffer supplemented with 125 μM ATP, 10 μM $MgCl_2$, 100 μM MAPKAP kinase 2 substrate peptide (Upstate Biotech.), and 10 μCi [γ-^{32}P]ATP (Amersham). The kinase reaction was allowed to proceed at 30°C for 30 min, after which 25 μL of each reaction mixture was spotted onto individual p81 phosphocellulose filters (Whatman, Clifton, New Jersey). These filters were washed with four changes of 0.75% phosphoric acid, once with acetone, air-dried, and then analyzed in a scintillation counter for ^{32}P.

Measurement of plasma concentration of SB242235 Approximately 0.5 mL of blood was collected at each time point by cardiac puncture. Plasma was isolated using a Microtainer tube with lithium heparin, according to the manufacturer's protocol (Becton Dickinson, Franklin Lakes, New Jersey). Quantification of SB242235 was conducted by high-pressure liquid chromatography with mass spectrophotometric detection following organic solvent extraction from plasma.

IL-6 and KC ELISA Extracts prepared from UVB irradiated and control skin were analyzed for IL-6 and KC ELISA (Mouse IL-6 ELISA, Ebioscience, San Diego, California, standards and samples diluted in PBS containing 1.0% FBS; Mouse KC ELISA, R&D Systems (Minneapolis, Minnesota), standards prepared in RD5-3 diluent with samples diluted 2 × in RD5-3 diluent). The assay was performed following the manufacturers' guidelines.

Statistical analyses Statistical analyses were performed using Student's *t* test (two-tailed): $p < 0.05$ was considered to be statistically significant.

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Address correspondence to: Arianna L. Kim, Department of Dermatology, Columbia University College of Physicians & Surgeons, 630 West 168th Street VC15-204, New York, New York 10032, USA. Email: ak309@columbia.edu

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