Direct and indirect modulation of ornithine decarboxylase and cyclooxygenase by UVB radiation in human skin cells

Marco Soriani, Patrick Luscher1 and Rex M.Tyrrell2

Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK and 1 Swiss Institute for Experimental Cancer Research (ISREC), Epalinges, Switzerland

2To whom correspondence should be addressed
Email: prsrmt@bath.ac.uk

Exposure to solar ultraviolet (UV) B radiation is responsible for skin inflammation and tumour progression. Cyclooxygenase and ornithine decarboxylase are believed to be involved in such processes since they participate in the synthesis of mediators of inflammation and cell differentiation, respectively. We have investigated the in vitro modulation of expression of such genes by UVB radiation in different skin cell lines. We have observed that accumulation of ornithine decarboxylase mRNA is unaffected by even high UVB doses in both human epidermal keratinocytes and dermal fibroblasts, whereas cyclooxygenase-2 levels were significantly up-regulated by low UVB doses in KB human epidermoid keratinocytes. Depletion of total intracellular glutathione levels in KB cells amplified the activation, revealing a role for an oxidative component of UVB in modulating cyclooxygenase gene expression. Transfer of medium from UVB irradiated keratinocytes to fibroblasts resulted in a significant activation of cyclooxygenase expression and activity, while ornithine decarboxylase levels were unaffected. We conclude that UVB radiation can activate cyclooxygenase gene expression in human skin cells both by direct activation pathways or indirectly by inducing a paracrine mechanism.

Introduction

Increased exposure to the ultraviolet (UV) B (290–320 nm) component of sunlight has been associated with an enhancement in the incidence of skin cancer (1). UVB radiation not only acts by inducing DNA damage and cell mutagenicity, but can also modulate the expression of several genes at dose levels relevant to natural solar exposure (2). Some of these genes are believed to be regulated by an oxidative mechanism (3,4). In the present study we have investigated the effect of UVB radiation on the in vitro regulation of two important enzymes that participate in inflammation and cancer progression: ornithine decarboxylase (ODC) (5) and cyclooxygenase (Cox) (6). Both polyamines and prostaglandins, ODC and Cox products, respectively, are associated with malignant transformation (7,8). In particular, polyamines, by regulating cell growth and differentiation, have been classified as tumour promoters (9), growth factors (10) and UV radiation (12–15). Although there is some evidence both for and against a role of an oxidative component in UVB activation of ODC (16,17), in vivo activation of ODC appears to be maximal after short wavelength (290 nm) UVB radiation (18). Several in vivo studies have demonstrated a relationship between UVB radiation and increases in ODC activity levels in the skin of mice (12–14) and humans (19). On the other hand, only two in vitro studies performed on newborn rat keratinocytes have provided an indication of a direct activation of ODC by UVB radiation (15,20).

Induction of ODC activity in mouse skin by tumour promoters appears to be mediated by products of Cox activity (21,22). In contrast, the induction of ODC in human epidermis is independent of lipoxigenase and Cox pathways (19). Cox is a heme-containing enzyme that plays an important role in inflammation and cancer progression (23). Cox catalyses the oxidation of arachidonic acid (AA) into inflammatory mediators such as prostaglandins and is encoded by two related genes, Cox-1 and Cox-2 (24,25). The Cox-1 gene is constitutively and ubiquitously expressed, while the Cox-2 gene is only expressed at high levels following induction by growth factors (26), cytokines (27–29) and extracellular stimuli, such as UVB radiation (29). Since both ODC and Cox are believed to be involved in UVB-mediated skin damage, we have investigated the mechanism of activation of such genes by UVB radiation in human skin cells in vitro.

Culture medium from UV-irradiated dermal fibroblasts has been shown to contain a series of growth factors that indirectly activate gene expression in non-irradiated fibroblasts (30,31). In this study we have investigated the role of UV-induced factors in transducing gene activation signals from UVB-irradiated epidermal keratinocytes to non-irradiated dermal fibroblasts. UVB radiation can up-regulate a wide range of cytokines, including the interleukins (IL) IL-1, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α) and granulocyte/macrophage colony-stimulating factor in both cultured human keratinocytes and epidermis in vivo (32–37). Moreover, a recent study by Kondo et al. (38) shows that keratinocyte-derived cytokines released following UVB irradiation are available for interactions below the basement membrane. It is therefore possible that infiltrating soluble cytokines released by keratinocytes could up-regulate gene expression in dermal fibroblasts by a paracrine mechanism.

In the present in vitro study, we have investigated the ODC and Cox-2 gene response to UVB radiation in human skin cells and the possible involvement of a paracrine mechanism in the UVB modulation of expression of these genes.

Materials and methods

Chemicals

Cell culture media and serum were purchased from Fakola (Basel, Switzerland). All chemicals and biochemicals where not otherwise indicated were obtained from Sigma (St Louis, MO).
Cell strains and culture

The human skin fibroblast cell line FEK4 was derived from a foreskin explant and cultured as described before (39). The human carcinoma cell line KB (29) was kindly provided by Prof. Jean Krutmann (Heinrich-Heine-University, Dusseldorf, Germany). The spontaneously immortalized human keratinocyte cell line HaCat (40) was kindly provided by Dr Peter Beard (ISREC, Epalinges, Switzerland) and cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS). The human transformed fetal keratinocyte cell line HFK SV61 (41) was grown in the same medium as FEK4, except that 10% FCS was added and that the medium was supplemented with 40 μg/ml hydrocortisone.

Cell treatments

Cells grew in a monolayer up to 70–80% confluence. For UVB radiation treatment medium was removed and cells were rinsed in isotonic phosphate-buffered saline (PBS). During irradiation cells were covered with a solution of PBS containing CaCl2 and MgCl2. UVB radiation was provided by a bank of Philips TL 40W/01 experimental lamps with an irradiance of 4.5 W/m² (emission peak between 310 and 315 nm). The irradiance was monitored with an IL1700 radiometer. Cells were irradiated through the plastic cover of the culture dish, to cut off any contaminating UVC radiation. After irradiation, conditioned medium was replaced and cell populations were incubated for various periods of time prior to RNA extraction. Survival of colony-forming ability after UVB was measured as described previously (42).

In order to deplete intracellular glutathione (GSH), 20 μM D,L-buthionine-S,R-sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthase, was added to the culture medium 18 h prior to UVB radiation (43).

In transfer medium experiments, keratinocytes were grown in 200 mm dishes and treated with UVB at 60–70% confluence. After irradiation, fresh low serum medium (0.5 or 2% FCS) was added to avoid any non-specific induction of cell metabolites by factors contained in the serum. After 24 h the medium was collected and transferred to 90% confluent fibroblasts pre-cultured overnight in low serum medium. Total fibroblast RNA was extracted at various time points and analysed for evidence of modulation of ODC and Cox-2 gene expression.

Isolation and analysis of total RNA

Total RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform extraction method and subjected to northern analysis. Twenty-five micrograms of total RNA were then loaded onto a MOPS/formaldehyde agarose gel (1.3%), electrophoresed and transferred to a sheet of Zeta-Probe (Bio-Rad, Hercules, CA) and hybridized to a 32 P-labelled cDNA probe. 32 P-labelled cDNA probes for ODC and Cox-2 were prepared (Molecular Dynamics, Sunnyvale, CA) to perform densitometry analysis.

Glutathione measurements

Total intracellular GSH and the oxidized form, glutathione disulfide, were isolated by TCA/EDTA extraction and spectrophotometrically determined as previously described (43).

Cox activity measurements

The Cox activity assay was based on a method previously described by Grewe et al. (44). After UVB radiation treatment, cells were disrupted, incubated with [14C]AA (Amersham, Germany) and resulting metabolites were extracted and analysed by HPLC. The HPLC system was from Kontron Instruments (Zurich, Switzerland), equipped with an HPLC 360 autosampler. Samples were separated on a Waters NovaPak reverse phase C18 column (0.39 x 15 cm) (Waters, Milford, MA) using an acetonitrile/trifluoroacetic acid gradient, at a flow rate of 1.5 ml/min.

Results

Effect of UVB radiation on ODC gene expression in human epidermal cells

To determine the capacity of UVB radiation to directly modify ODC gene expression in vitro we tested several human skin cell lines under different irradiation conditions. FEK4 dermal fibroblasts, HFK SV61, HaCaT and KB transformed keratinocytes did not show any significant induction of ODC expression at relatively low UVB radiation doses (50–500 J/m²) at time points between 3 and 24 h. In KB cells, doses up to 1 kJ/m² were ineffective in up-regulating ODC levels. However, a well-known ODC inducing agent, 12-O-tetradecanoylphorbol-13-acetate (TPA), up-regulated ODC gene expression in all the cell lines tested (data not shown). Figure 1 shows that in cloning efficiency tests performed on UVB-irradiated KB cells, ~80% of cells survived UVB doses of 500 and 1000 J/m².

UVB regulation of Cox-2 gene expression in human keratinocytes

KB transformed human keratinocytes (KB cells) exposed to UVB radiation doses of 250 and 500 J/m² showed a significant but variable induction of Cox-2 gene expression (Figure 2). A 2- to 3-fold maximal Cox-2 induction was observed between 3 and 8 h post-irradiation. The Cox-2 gene activation observed at a UVB radiation dose of 500 J/m² was associated with an increase in prostaglandin synthesis of 22.3 ± 4.0% (n = 3) at 24 h post-irradiation. We also tested Cox-2 inducing agents including TPA, epidermal growth factor and TNF-α as positive controls for the responsiveness of KB cells to external stimuli. Only TPA showed a major induction of Cox-2 expression (up to 25-fold) and enzymatic activity (prostaglandin synthesis increased by 343.3 ± 182.0%, n = 3) in KB cells.

In order to verify the role of the oxidative component of UVB on such an induction, we have investigated the influence of cellular GSH status on Cox-2 gene activation by UVB radiation. Eighteen hours of incubation of KB cells with 20 μM BSO resulted in an ~65% depletion of intracellular GSH. Further increases in BSO concentration up to 100 μM did not significantly increase the effect. BSO treatment enhanced UVB radiation-mediated Cox-2 expression in KB human epidermoid keratinocytes. Figure 3 shows that the effect of 500 J/m² UVB radiation dose on Cox-2 expression was increased up to 2-fold in glutathione-depleted KB cells. Moreover, incubation with BSO did not significantly alter the basal level of Cox-2 gene expression in KB cells (Figure 3a).

UVB radiation doses of up to 4 kJ/m² did not significantly reduce total intracellular GSH concentration in KB cells, whereas UVA (250 kJ/m²)-irradiated KB cells showed an ~20% decrease in total GSH (43).
ODC and Cox-2 modulation by UVB

Fig. 2. Effect of UVB radiation on Cox-2 mRNA accumulation in KB cells. Cells were UVB irradiated at (a) 250 and (b) 500 J/m². Black columns represent sham-irradiated control cells and grey columns UVB-irradiated cells. The results are the means ± SD of three independent experiments. Northern blots (c) show typical patterns of Cox-2 and GAPDH mRNA expression in UVB-irradiated KB cells at 250 and 500 J/m². Total RNA was collected 3, 4 and 8 h post-irradiation.

Regulation of human fibroblast Cox-2 and ODC levels by soluble factors released by human keratinocytes.

Recently, it has been demonstrated that soluble cytokines released by keratinocytes upon UVB radiation can penetrate through the basal membrane of epidermis and infiltrate into the dermis (38). This mechanism could be responsible for paracrine regulation of fibroblast gene expression. Figure 4 shows that medium collected from UVB-irradiated KB keratinocytes is able to up-regulate Cox-2 gene expression in human fibroblasts up to 6-fold with respect to Cox-2 basal level and 3-fold with respect to cells incubated in non-irradiated keratinocyte medium. Comparable results were obtained by transferring medium from UVB-irradiated HaCat human keratinocytes (Figure 5). This induction of Cox-2 mRNA accumulation was associated with an increase in Cox enzymatic activity. Fibroblasts incubated for 24 h in medium taken from UVB-irradiated KB cells showed a 41.1 ± 14.6% (n = 3) increase in prostaglandin production with respect to Cox-2 basal level and 24.1 ± 13.2% (n = 3) with respect to cells incubated in non-irradiated keratinocyte medium. The effect of factors released following UVB irradiation of keratinocytes on ODC gene expression was also tested. No significant differences were observed in levels of ODC mRNA accumulation between human fibroblasts treated with medium derived from UVB-irradiated keratinocytes and untreated control cells (data not shown).

Discussion

In the present study we have observed that physiological doses of UVB radiation did not activate the expression of ODC in human skin cells, but caused a significant induction of Cox-2 expression (Figure 2) in human keratinocytes. Since in vivo (14,18) and in vitro (15) studies showed that UVB radiation induced ODC mRNA expression and activity in rodents, the
lack of an increase in ODC expression in human skin cells reported in this study indicates that UVB radiation modulates ODC expression by different pathways in rodents and humans. A further explanation for this difference is that the irradiation conditions used by our group differ from those in other laboratories. In all the experiments performed in this study, the UV radiation emitted from banks of Philips TL-01 UVB lamps was mainly in the UVB region between 310 and 315 nm (90% total energy) with a peak at 312 nm and was filtered through the lid of the Petri dish used to culture the cells to exclude UV wavelengths <300 nm. The levels of UVA radiation were not biologically significant. All the data previously described for in vitro UVB modulation of ODC expression were obtained with a Westinghouse FS-20 UVB lamp with a principal emission between 280 and 380 nm, peaking at 313 nm (20). Furthermore, Petri dish lids were removed prior to irradiation so that the UVB-mediated ODC activation observed in rat keratinocytes may be partially mediated by contaminating UVC radiation as well as UVB wavelengths shorter than those used here.

Both UVB and UVA radiation can induce AA metabolism (3,45). In particular, physiological doses of UVB radiation increase prostaglandin synthesis by up to 2-fold in transformed human keratinocytes (29). In this study Cox-2 RNA levels were up-regulated by UVB radiation at physiologically relevant doses and we observed a maximum induction of 2- to 3-fold between 3 and 8 h post-irradiation (Figure 2). A recent paper from Buckman et al. (46) shows that in human keratinocytes, UVB induced Cox-2 mRNA expression up to 12-fold, at 6–12 h post-irradiation. This apparent discrepancy could be related to the fact that they used primary keratinocytes 2–3 days after they had reached confluency, i.e. in arrested growth conditions, and a different UVB lamp (Westinghouse FS-20). In our system, KB cells were grown for 2–3 days and at the time of irradiation were 60–70% confluent. We used such conditions since at confluency >80%, cells started to detach from the culture plate. However, our observation that UVB radiation modulates Cox-2 expression has been obtained in human transformed keratinocytes and the intensity of the response does not necessarily apply to primary keratinocytes.

It has been shown that in human keratinocytes, UVB
radiation-induced up-regulation of cytoplasmic phospholipase A₂ and prostaglandin synthesis is mediated by UVB radiation-induced formation of free radicals (3). Consistent with a role of the oxidative component of UVB radiation in modulation of Cox-2 gene expression, N-acetylcyesteine, a precursor of GSH synthesis, inhibited UVB-induced prostaglandin synthesis in human keratinocytes (47). Our data showing that intracellular GSH depletion potentiates UVB radiation-mediated Cox-2 induction (Figure 3) further substantiates this model. Moreover, the negligible consumption of total intracellular GSH levels by UVB radiation in KB cells is consistent with in vivo studies showing that after large UVB radiation doses total GSH levels were only slightly affected by UVB radiation in mouse skin homogenates (48).

Recently it has been postulated that keratinocyte-derived cytokines could affect the response of distal skin areas by crossing the basement membrane that separates epidermis from dermis (38). Keratinocytes are a major source of cytokines in the epidermis and UVB radiation clearly up-regulates IL-1 (49), IL-6 (50) and TNF-α (51) levels. Less clear is how such factors infiltrate into the dermis and trigger the cascade of events leading to both skin inflammation and cancer. Cyclooxygenase expression is modulated by several cytokines (52,53) and, in particular, keratinocyte prostaglandin synthesis is stimulated by IL-1 (28). Since UVB radiation leads to a large and diffuse inflammatory response in vivo, we have tested the possibility that Cox-2 activation in the dermis could be triggered by an indirect effect of UVB radiation mediated by factors released by epidermal keratinocytes. When culture medium containing soluble factors released by UVB radiation-stimulated keratinocytes is transferred to primary dermal fibroblasts we observe a significant increase in both Cox-2 mRNA accumulation (Figures 4 and 5) and activity (see Results) compared with control cells incubated with medium from unirradiated keratinocytes. We are now investigating which specific classes of cytokines released by HaCaT and KB cells are involved in Cox-2 activation in FEK4. The absence of ODC induction in FEK4 fibroblasts incubated with medium from UVB-irradiated keratinocytes may be related to the fact that ODC is differently regulated by cytokines. Indeed, IL-1 down-regulates ODC activity in human melanoma cells (54), while TNF-α stimulates ODC activity in human fibroblasts (55). Since in human keratinocytes, both IL-1 and TNF-α are up-regulated by UVB radiation (29,56), it is possible that they may both be produced by UVB-irradiated HaCaT or KB cells and then exert opposite effects on ODC expression in dermal fibroblasts. However, keratinocytes represent only a fraction of the cells participating in the skin immune system. In particular, tissue dendritic cells, including pigment forming melanocytes, Langerhans cells, tissue macrophages and dermal dendrocytes, all have a role in regulating the response of the skin to external stimuli (57,58) and they could also be involved in the UVB-mediated ODC activation observed in vivo.

In conclusion, we have demonstrated that in human skin cells, ODC mRNA expression is not modified as a result of direct effects of UVB radiation and that UVB radiation-mediated release of soluble factors from epidermal keratinocytes does not affect ODC expression in dermal fibroblasts. We have also confirmed that in human keratinocytes, Cox-2 expression is up-regulated by UVB radiation and that the mechanism of activation probably involves the oxidative component of UVB radiation. Moreover, we have provided evidence for an in vitro ‘paracrine’ activation of Cox-2 expression in dermal fibroblasts that is mediated by soluble factors released by UVB-irradiated epidermal keratinocytes.

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