Ultraviolet Radiation Induction of Ornithine Decarboxylase in Rat Keratinocytes

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ABSTRACT

Ultraviolet (UV) radiation plays an important role in the induction of cutaneous malignancy, including basal cell and squamous cell carcinomas and malignant melanoma. In addition to its effects on DNA damage and repair mechanisms, UV radiation has been shown to modulate the expression of specific genes, altering the levels of their mRNAs and the synthesis of their corresponding proteins. In order to gain further information about the molecular effects of UV radiation, we have studied the regulation of ornithine decarboxylase (ODC) gene expression in response to UV radiation. ODC is the rate-limiting enzyme in polyamine biosynthesis, is involved in growth and differentiation, and has been implicated in carcinogenesis. Keratinocytes grown in culture were either sham-irradiated or exposed to increasing doses of UVB (1–5 mJ/cm²). Northern blot analysis of keratinocyte RNA under basal conditions demonstrated the presence of two ODC mRNA transcripts. Increasing exposure to UVB resulted in a dose-dependent increase in the levels of both ODC mRNA transcripts. The induction of ODC gene expression following UVB exposure was noted 2 h after UVB exposure, and ODC mRNA levels continued to increase up to 24 h after UVB exposure. The UVB-induced increase in ODC gene expression was not dependent on serum alone, despite the ability of serum alone to induce ODC gene expression. The UVB-induced increase in ODC activity is due, at least in part, to an increase in ODC gene expression and they provide a useful model for the analysis of the molecular effects of UVB radiation.

INTRODUCTION

UVR produces multiple alterations in cutaneous biology, including inflammation, proliferation of keratinocytes and melanocytes, and induction and/or exacerbation of diseases of the skin. UVR has also been shown to be an environmental carcinogen, implicated in the development of cutaneous malignancy, including basal cell and squamous cell carcinoma and malignant melanoma. UV radiation has been arbitrarily divided into three regions according to wavelength: UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm). Although solar UV radiation contains UVA, UVB, and UVC, only UVA and UVB are present in terrestrial sunlight. Exposure of skin to these wavelengths can produce significant damage to cutaneous structures. Both UVA and UVB are capable of damaging DNA, resulting in the production of cyclobutane pyrimidine dimers and (6–4)thymine photoproducts. Furthermore, both UVA and UVB have been shown to be mutagenic in several model systems (1, 2).

Despite the accumulating body of evidence implicating UVR exposure in the pathogenesis of tissue injury and cutaneous disease, the molecular and cellular basis for the multiple effects of UV radiation remains incompletely understood. The results of recent experiments have suggested that UVR may exert its effects in part through the modulation of the transcriptional activity of specific genes. UVC, perhaps the best studied of the UVR wavelengths, has been shown to exert its effects at the levels of both mRNA translation and gene transcription (3–7). Considerably less information is available with regard to the specific mechanisms of action of UVA and UVB, the regions of the UV spectrum known to be important in the pathogenesis of cutaneous disease.

In order to gain further information about the molecular effects of UVB, we have been studying the UVB induction of the enzyme ODC in a newborn rat keratinocyte cell line (8, 9). ODC is the first enzyme in the mammalian polyamine biosynthetic pathway, forming putrescine by the decarboxylation of ornithine. The polyamines ( spermine, spermidine, and putrescine) play an important role in cell growth and differentiation and have been implicated in the process of carcinogenesis (10, 11). ODC enzyme activity has been shown to be induced by many stimuli, including androgens (3), serum (12), UVB radiation (13–15), c-Ha-ras oncogene transfection (16), polyamine deprivation (17), and phorbol esters (18, 19). The regulation of ODC activity has also been shown to occur at different levels, including ODC gene transcription (3, 4), regulation of mRNA stability, mRNA translation (5–7), and protein stability (16, 20, 21), and post-translational interactions and modifications (22, 23). We report that exposure of rat keratinocytes to UVB results in a dose-dependent increase in ODC activity, which is associated with a specific and dose-dependent increase in the expression of the ODC gene.

MATERIALS AND METHODS

Materials. Tissue culture media and calf and fetal bovine sera were obtained from Gibco (Burlington, Ontario) or Amersham Corp. (Oakville, Ontario). [α-32P]-ATP (>800 Ci/mmol) was from ICN Radiochemicals (Montreal, Quebec). Restriction enzymes and DNA polymerase were obtained from Pharmacia LKB Biotechnology Inc. (Montreal, Quebec). All chemicals were from Sigma (St. Louis, MO) or Fisher (Markham, Ontario). Nitrocellulose membranes were obtained from Schleicher and Schuell (Montreal, Quebec). The mouse ODC cDNA (pODC16) was kindly provided by Dr. O. Janne, The Population Council and Rockefeller University (New York, NY). The hexoseaminidase A cDNA was a gift from Dr. R. G. Korneluk and Dr. J. A. Lowden, Hospital for Sick Children, University of Toronto, Toronto, Ontario. The chicken actin cDNA was obtained from Dr. D. Cleveland, Johns Hopkins University (Baltimore, MD).

Cell Culture. Newborn rat keratinocytes were kindly provided by Dr. H. Baden (Massachusetts General Hospital, Boston, MA) (9). These cells continue to produce several macromolecules characteristic of differentiated keratinocytes, such as desmosomes and cornified envelopes. They have not undergone malignant transformation, inasmuch as they did not form colonies when inoculated onto soft agar and did not produce tumours on inoculation into newborn rats (9). Cells were grown in Dulbecco’s minimal essential medium, supplemented with 1% hydrocortisone and either 5% fetal bovine serum and 10% calf serum (designated regular serum) or 0.05% fetal bovine serum and 0.1% calf serum (designated low serum). Cells were grown under an atmosphere of 5% CO2 at 37°C. Unless otherwise noted, experiments were carried out on cells passaged fewer than 20 times.
UV Irradiation. The light source consisted of two Westinghouse FS-20 bulbs, which emit UV radiation between 280 and 380 nm with a principal emission between 290 and 320 nm, peaking at 313 nm. Irradiance measurements were determined prior to each experiment using an IL-500 radiometer with a SEE-240 UVB probe, with an IL-UVB filter. At least four irradiance measurements were taken in each field site to ensure uniform irradiance, within a range of ±10%, before each set of exposures. The mean irradiance was 0.05 mW/cm². The distance between the light source and the probe was the same as that between the source and the tissue culture plates during irradiation. Immediately prior to irradiation, the culture medium was removed and replaced with phosphate-buffered saline. The plastic lids were removed from the tissue culture plates for the duration of the irradiation. Measurement of medium temperature demonstrated less than a 0.1°C change in temperature following irradiation. After irradiation, the saline was replaced with culture media containing either regular or low serum as specified. Sham-irradiated keratinocytes were treated identically but not exposed to UV.

Assay of ODC Activity. For the determination of ODC activity, keratinocytes were rinsed with phosphate-buffered saline and scraped with a rubber policeman into 1.5 ml of ODC assay buffer, consisting of 50 mM Tris-HCl, 1 mM EDTA, and 15 mM dithiothreitol, at pH 7.8. Cells were sonicated while on ice and centrifuged for 1 h at 15,000 x g at 4°C. ODC activity in the clear supernatant was determined by measuring the release of 14CO2 from L-[1-14C]ornithine, as previously described (14). The assay mixture contained 50 mM Tris-HCl, 1.0 mM EDTA, 0.1 mM pyridoxal phosphate, 15 mM dithiothreitol, 0.4 mM L-ornithine, and 0.4 μCi L-[1-14C]ornithine, including 188 μl of the supernatant in a final volume of 200 μl. The reaction was carried out in 15-ml snap-top tubes (Falcon), in which a paper disc (Schleicher and Schuell, Keen, NH) soaked in toluene and NCS (Amersham) was suspended on a 22-gauge needle. Each sample was incubated at 37°C for 60 min, with the released 14CO2 being absorbed onto the paper disc. The reaction was stopped by the addition of 0.5 ml of 2 M citric acid. The incubation was then continued for a further 60 min to allow for complete absorption of the 14CO2. The discs were then transferred to vials containing 10 ml Omnifluor (DuPont). Radioactivity was measured in a Beckman liquid scintillation counter. Assays were always carried out in triplicate. Protein content was determined using the Bradford assay (24), with bovine serum albumin as the standard.

RNA Analysis. Total cellular RNA was isolated as previously described (25). RNA was size fractionated by formaldehyde-agarose gel electrophoresis, stained with ethidium bromide to assess the integrity of the preparation, gel scanned, and migration of the RNA, and transferred to a nitrocellulose membrane (Schleicher and Schuell). The RNA was fixed to the membrane by UV irradiation. cDNA probes for mouse ODC, chicken actin, and human hexoseaminidase A were labeled by the random priming technique, using a kit from BRL. Hybridization was performed in the same solution with 1 x 10⁶ cpm/ml 32P-labeled cDNA probe, for 24 h at 42°C. Final washes were 0.1x saline sodium citrate, 0.1% sodium dodecyl sulfate, at 50°C. Autoradiography was performed using Kodak X-Omat film at −70°C. Autoradiograms were quantified by scanning with a laser densitometer.

RESULTS

To define an appropriate dose range of UVB exposure for the analysis of the UVB induction of ODC enzyme activity, we examined the effects of UVB radiation on the growth of keratinocytes in vitro. Following exposure of keratinocyte cultures to 6 mJ/cm² or greater, more than 80% of the cells did not survive more than 24 h. After exposure to 5 mJ/cm², 70% of cells survived, while, after receiving a dose of 2 mJ/cm², 90% of cells remained viable. Accordingly, we examined the effects of a range of UVB doses, varying from 1 to 5 mJ/cm², on the induction of ODC activity in subconfluent 10-cm plates of rat keratinocyte cultures (2-3 x 10⁶ cells/plate). Cells were harvested for the measurement of ODC activity 24 h following exposure to UVB. The results of these experiments are shown in Fig. 1. A significant increase in ODC enzyme activity was noted following exposure of keratinocytes to only 1 mJ/cm², the lowest dose of UVB tested. Exposure of keratinocytes to UVB doses of 2, 3, and 4 mJ/cm² resulted in approximately 3-fold increases in ODC enzyme activity, whereas exposure to 5 mJ/cm² produced a 5-fold increase in ODC activity. Previous studies have shown a good correlation between ODC enzyme activity and the amount of enzyme present, as quantified by radioimmunoassay (10, 20, 27). In view of the known effects of serum on the induction of ODC enzyme activity in other cell lines (12), we analyzed the effects of serum on the UVB-induced increase in ODC enzyme activity in rat keratinocyte cultures. The increase in ODC activity following UVB exposure was detected both in cells incubated in medium containing regular serum, as depicted in Fig. 1, and in cells incubated in medium supplemented with low serum (see "Materials and Methods") concentrations (data not shown).

To determine whether the UVB-induced increase in ODC enzyme activity was associated with an increase in ODC gene expression, total cellular RNA from irradiated and sham-irradiated keratinocytes was isolated 24 h following exposure to different doses of UVB. Northern blot analysis of keratinocyte RNA demonstrated the presence of two distinct ODC mRNA transcripts, approximating 2.2 and 2.7 kilobases in size (Fig. 2A). Two distinct ODC mRNA transcripts have been demonstrated in a number of different tissues (19, 28, 29) and have been shown to differ from each other in the 3′-untranslated region of the ODC mRNA transcript (30). An increase in the levels of both ODC mRNA transcripts was seen after exposure to the smallest dose of UVB tested, 1 mJ/cm². The levels of ODC mRNA transcripts increased progressively following exposure to increasing doses of UVB. An approximately 7-fold increase above control was noted with the two highest doses tested, 4 and 5 mJ/cm² (Fig. 2A and C). To determine the relative specificity of the UVB-induced increase in ODC gene expression, we examined the effects of UVB radiation on the expression of two additional genes. The Northern blot depicted in Fig. 2A was rehybridized sequentially with cDNA probes for actin, a constitutively expressed housekeeping gene, and hexoseaminidase A, a gene which encodes a lysosomal enzyme.

Fig. 1. Effect of increasing doses of UVB on ODC activity in rat keratinocytes. Keratinocytes in culture were irradiated with UVB (1–5 mJ/cm²) or sham irradiated (0 mJ/cm²). ODC activity was measured 24 h after irradiation and is reported as nmol/CO₂/mg protein/h ± SE. Each value represents the mean of three separate experiments.

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was relatively specific and not a generalized cellular response constant, even after exposure of keratinocytes to doses of UVB as high as 5 mJ/cm². The results of these experiments demonstrated that the UVB-induced increase in ODC gene expression was relatively immediate after sham irradiation, following which they declined to lower control levels by 12 h. In view of the well known stimulatory effect of serum on ODC enzyme activity and ODC mRNA levels in different cells (12) and since the sham-irradiated cells were replenished with fresh medium containing serum, we examined whether serum alone increased the expression of the ODC gene in rat keratinocytes. Cells were preincubated under low serum conditions for 12 h, following which half the cells were then exposed to UVB radiation (3 mJ/cm²). After irradiation, half the cells were continued in low serum, whereas the other half were switched to medium containing regular serum. Sham-irradiated controls were treated in an identical fashion. Sham-irradiated control cells which were switched to regular serum had slightly higher (1.3–1.6-fold) levels of ODC mRNA transcripts (Fig. 4; 12 h time point shown). This effect of serum was also detected in UVB-irradiated cells. However, the increase in the levels of ODC mRNA transcripts following UVB radiation was detected in cells grown in either regular or low serum. These observations demonstrated that the UVB induction of ODC gene expression was relatively independent of the serum concentration present in the medium.

DISCUSSION

UV radiation has been shown to induce ODC enzyme in keratinocytes, both in vivo and in vitro. Studies in mice have shown that epidermal ODC activity increases following both single and multiple UVB exposures, in a dose-dependent manner (13). The increase in ODC activity occurred prior to the increase in DNA synthesis, with a significant increase in ODC enzyme activity noted 2 h after irradiation and a peak at 24–28 h (14). The induction of ODC activity was found to be wavelength dependent in the intact mouse, with peak effectiveness occurring at 290 nm (15, 31). No stimulation of ODC activity was observed following exposure to 315 nm. These observations suggested that the interactions between UVR and the subsequent induction of ODC activity might provide a useful model for the study of the molecular effects of UV radiation.

The effects of UVB exposure on the levels of actin and hexoseaminidase mRNA transcripts were shown in Fig. 2, A and B, respectively. Only a small (<1.5-fold) increase in actin mRNA transcripts was detected following UVB exposure. In contrast, the levels of hexoseaminidase A mRNA transcripts remained constant, even after exposure of keratinocytes to doses of UVB as high as 5 mJ/cm². The results of these experiments demonstrated that the UVB-induced increase in ODC gene expression was relatively specific and not a generalized cellular response to UVB radiation.

To delineate the time course of the UVB-induced increase in the levels of ODC mRNA transcripts, keratinocytes were irradiated with 3 mJ/cm² and total cellular RNA was isolated at multiple time points following exposure to UVB (Fig. 3). Total cellular RNA was also isolated from control sham-irradiated cells at each time point. An increase in ODC mRNA levels following UVB exposure was detected at 2 h, the first time point studied. Maximum UVB induction of ODC mRNA levels was seen by 12 h, and the amounts of ODC mRNA transcripts remained relatively constant through 48 h (48 h time point not shown). In contrast to the rapid and time-dependent increase in the levels of ODC mRNA transcripts following UVB exposure, no significant changes in the levels of either actin (Fig. 3) or hexoseaminidase A (data not shown) mRNA transcripts were detected under the same experimental conditions.

The results of the experiment shown in Fig. 3 indicated that the levels of mRNA transcripts in control cells tended to be consistently higher immediately after sham irradiation, following which they declined to lower control levels by 12 h. In view of the well known stimulatory effect of serum on ODC enzyme activity and ODC mRNA levels in different cells (12) and since the sham-irradiated cells were replenished with fresh medium containing serum, we examined whether serum alone increased the expression of the ODC gene in rat keratinocytes. Cells were preincubated under low serum conditions for 12 h, following which half the cells were then exposed to UVB radiation (3 mJ/cm²). After irradiation, half the cells were continued in low serum, whereas the other half were switched to medium containing regular serum. Sham-irradiated controls were treated in an identical fashion. Sham-irradiated control cells which were switched to regular serum had slightly higher (1.3–1.6-fold) levels of ODC mRNA transcripts (Fig. 4; 12 h time point shown). This effect of serum was also detected in UVB-irradiated cells. However, the increase in the levels of ODC mRNA transcripts following UVB radiation was detected in cells grown in either regular or low serum. These observations demonstrated that the UVB induction of ODC gene expression was relatively independent of the serum concentration present in the medium.
ULTRAVIOLET B RADIATION INDUCTION OF ODC

Fig. 3. Northern blot analysis of time-dependent increases in ODC mRNA transcript levels following UVB radiation. Keratinocytes grown in low serum medium were exposed to 3 mJ/cm² UVB or sham-irradiated (C). Total cellular RNA was isolated at 2, 6, 12, and 24 h, and 10 µg of RNA were used for Northern blot analysis. The blot was hybridized with cDNAs for ODC and actin (A) and exposed for 12 h.

Fig. 4. Effect of serum on basal (C) and UV-stimulated levels of ODC mRNA transcripts. Keratinocyte cultures were grown in low serum medium for 12 h prior to irradiation. After irradiation with 3 mJ/cm² UVB, one group (LS) was returned to low serum medium. The other group (RS) was subsequently grown in medium containing regular serum after irradiation. Twelve h following exposure to UVB, total cellular RNA was isolated and analyzed by Northern blot analysis with a cDNA probe for ODC.

Constitutively elevated levels of ODC activity and mRNA transcripts (18). Compounds which inhibited the induction of ODC activity by TPA (indomethacin and retinoic acid) were also found to inhibit the formation of murine skin tumors (32, 33). Taken together, these observations suggest a possible role for ODC in the progression to cutaneous neoplasia.

Additional insights into the control of ODC gene expression have come from studies examining the effect of serum and mitogens on the levels of ODC mRNA transcripts. Both serum stimulation and treatment with mitogens and TPA resulted in an increase in the transcription of the ODC gene in mouse fibroblasts (4). Withdrawal of serum from BC3H1 muscle cells led to a rapid decrease in ODC gene transcription; mitogenic stimulation or protein synthesis inhibition rapidly reinduced ODC mRNA levels in these cells, due to an increase in ODC gene transcription. In contrast, different results were obtained from studies of ODC gene transcription using mouse fibroblasts. Inhibition of protein synthesis strongly inhibited the serum-induced increase in ODC gene transcription, despite the concomitant superinduction of mRNA transcripts for the proto-oncogenes c-fos and c-myc (4). The UVR induction of the expression of certain nuclear oncoproteins, including c-myc and c-fos, in human keratinocytes also provides additional evidence for a close link between the cellular response to UVR and the regulation of cellular proliferation (34).

A number of different studies have examined the effects of UV radiation on the regulation of gene expression. Irradiation of primary human keratinocyte cultures with UVC (50 J/m²) produced small but significant qualitative and quantitative changes in protein synthesis (35). Total mRNA synthesis was reduced, although Northern blot analysis revealed a highly selective 2- to 5-fold increase in the level of specific mRNAs (36). Further characterization of the identity of these UVC-induced mRNA species has identified a group of keratins (36) and small proline-rich proteins (35) as members of a UV-inducible gene family. These studies demonstrated that UVC is capable of inducing protein synthesis and increasing mRNA levels in a highly selective manner. Among the proteins induced by UVC, at least two have been shown to be secreted proteins capable of inducing the synthesis of the UVR-induced group of proteins and mRNAs in previously unexposed cells (37, 38). UVC has also been shown to activate a quiescent gene coding for metallothionein-1 in murine lymphoma cells, conferring cadmium resistance. The fully expressed gene was found to have been extensively demethylated after UVC exposure, suggesting that UV-induced gene transcription may be associated with altered DNA methylation (39).

Much less is known about the effects of UVA and UVB on the regulation of gene expression. UVA has been shown to induce the synthesis of a Mr 32,000 protein in normal human fibroblasts, but the mechanisms responsible for this induction remain unknown (40, 41). Exposure of human keratinocytes to either UVA or UVB was much less effective than UVC in inducing the expression of the c-H-ras and c-myc genes in human keratinocytes (34). In contrast, human keratinocytes
exposed to UVB produced increased amounts of interleukin 1 activity, which were associated with increased amounts of interleukin 1 mRNA transcripts (40). The doses of UVB which were used in the latter study were much higher than those used in our experiments and may be due to the irradiation protocol, which involved irradiating the cells through one layer of tissue culture plastic in medium supplemented with 20% serum. Under these conditions, the effective dose of UVB reaching the cells would almost certainly be reduced, necessitating higher doses. The possibility that human keratinocyte cultures may be more radioresistant than the newborn rat keratinocyte cells used in the present study cannot be excluded.

The results of our studies demonstrate that increased expression of the ODC gene accounts, at least in part, for the increase in ODC activity following UVB exposure. Whether UVB radiation results in an increase in ODC gene transcription and/or a change in the stability of ODC mRNA transcripts is not possible to infer from our current experiments. We propose that exposure of mammalian cells to UV may result in the activation of a subset of cellular genes by stimulating the interaction of specific trans-acting factors with cis-acting UV response elements in the regulatory regions of UV-inducible genes. Further analysis of the molecular mechanisms which underlie the UVB-induced increase in ODC gene expression in rat keratinocytes should increase our understanding of the cellular effects of UV and may provide a useful model system for the study of the molecular effects of UV radiation.

REFERENCES


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