Ultraviolet B radiation induction of ornithine decarboxylase gene expression in mouse epidermis

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The cellular effects of u.v. radiation have been studied by using a hairless-mouse model *in vivo*. U.v. B radiation (u.v.B) induced the activity of the enzyme ornithine decarboxylase (ODC) in mouse epidermis. Maximal induction was noted after radiation with 90 mJ/cm², and increased ODC activity was first detected 2 h after u.v.B exposure. U.v.B. also induced the expression of the ODC gene in a time- and dose-dependent manner, but did not induce the levels of actin mRNA transcripts. Cycloheximide treatment did not alter basal levels of ODC mRNA transcripts and had no effect on the u.v.B induction of ODC-gene expression. The results of these experiments demonstrate that u.v.B radiation induces both the expression of the ODC gene and the activity of the enzyme, and provides a useful '*in vivo*' paradigm for the analysis of the molecular effects of u.v.B radiation.

INTRODUCTION

A large body of evidence has implicated u.v. radiation (u.v.r.) in the pathogenesis of tissue injury and cutaneous disease. Despite much interest in the cellular perturbations which arise as a consequence of exposure to u.v.r., the molecular and cellular basis for the multiple effects of u.v.r. remains incompletely understood. Recent work suggests that u.v.r. may exert its effects in part through the modulation of the expression of specific genes, both at the level of gene transcription and mRNA translation (Lieberman *et al.*, 1983; Kartasova *et al.*, 1987; Kupper *et al.*, 1987; Rotem *et al.*, 1987; Kartasova & van de Putte, 1988; Ronai *et al.*, 1988; Keyse & Tyrell, 1989). The u.v.r. regulation of gene expression appears to be complex, and has been shown to be mediated via a number of different signal-transduction pathways (Mai *et al.*, 1989).

U.v.r. has been arbitrarily divided into three regions according to wavelength; u.v.A (320–400 nm), u.v.B (290–320 nm) and u.v.C (200–290 nm). Although solar u.v.r. contains u.v.A, u.v.B and u.v.C, only u.v.A and u.v.B are present in terrestrial sunlight. Despite the importance of u.v.A and u.v.B in the pathogenesis of cutaneous disease, much of the work directed at understanding the molecular effects of u.v.r. has focused on u.v.C as an energy source. Much less is known about the cellular perturbations induced by u.v.B. In order to gain further information about the molecular effects of u.v.B irradiation (290–320 nm), we have been studying the u.v.B induction of the enzyme ornithine decarboxylase (ODC) in the hairless Skh mouse. ODC is the first enzyme in the mammalian polyamine-biosynthetic pathway, forming putrescine by the decarboxylation of ornithine. The polyamines spermine, spermidine and putrescine are known to be important in cell growth and differentiation and have been implicated in the process of carcinogenesis (Pegg & McCann, 1982; Pohjanpelto *et al.*, 1985).

ODC enzyme activity has been shown to be induced in murine epidermis by many stimuli, including u.v.r. Analysis of the different wavelengths comprising u.v.r. has shown that both u.v.B (Verma *et al.*, 1979) and u.v.C (Lowe, 1981) induce ODC

activity, whereas u.v.A is able to do so only in combination with phototoxic compounds (Gange, 1981). Thus, although both u.v.B and u.v.A are present in terrestrial sunlight and are capable of producing alterations in cutaneous biology, only u.v.B induces ODC activity.

We have shown that the u.v.B induction of ODC enzyme activity is associated with an increase in ODC-gene expression in rat keratinocytes *in vitro* (Rosen *et al.*, 1990). To extend these observations to a more physiologically relevant '*in vivo*' model of cutaneous biology, we have studied the effects of u.v.B on ODC enzyme activity and gene expression in hairless-mouse epidermis. We now report that exposure to u.v.B irradiation results in a marked increase in epidermal ODC activity, which is accounted for in part by an increase in the levels of ODC mRNA transcripts.

MATERIALS AND METHODS

Animals

Female Skh hairless mice, 6–8 weeks of age, were obtained from Charles River Canada (St. Constant, Quebec, Canada). They were allowed free access to water and Laboratory Rodent Chow (Purina).

Materials

[α -³²P]ATP (> 800 Ci/mmol) was from ICN Radiochemicals (Montreal, Quebec, Canada). Restriction enzymes and DNA polymerase were from Pharmacia LKB Biotechnology (Montreal, Quebec, Canada). All chemicals were from Sigma (St. Louis, MO, U.S.A.) or Fisher (Markham, Ont., Canada). Nitrocellulose membranes were obtained from Schleicher and Schuell (Montreal, Quebec, Canada). The mouse ODC cDNA (pODC16) was kindly provided by Dr. O. Jänne of The Population Council and Rockefeller University, New York, NY, U.S.A. The chicken actin cDNA was obtained from Dr. D. Cleveland, Johns Hopkins University, Baltimore, MD, U.S.A.

Abbreviations used: u.v.r., u.v. radiation; u.v.A, u.v.B and u.v.C, u.v. A, B and C (radiation); ODC, ornithine decarboxylase; P-5-P, pyridoxal 5-phosphate; DTT, dithiothreitol; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

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U.v.r.

The light source consisted of two Westinghouse FS-20 bulbs, which emit u.v.r. between 280 and 380 nm, with a principal emission between 290 and 320 nm, peaking at 313 nm. Irradiance measurements were determined before each experiment using an IL-1700 radiometer with an 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 $\pm 10\%$ before each set of exposures. The mean irradiance was 0.3 mW/cm^2 . The distance between the light source and the probe was the same as that between the source and the mice during irradiation. The mice were irradiated in specially constructed wire cages.

Assay of ODC activity

For the determination of ODC activity, mice were killed by cervical dislocation and the dorsal skin was excised. Immediately after excision, epidermis was separated by a brief heat treatment (30 s at 55°C), followed by exposure to 0°C for 90 s (Marrs & Voorhees, 1971). The skin was then spread flat and the epidermis removed by gently scraping with a no. 10 blade. The epidermis was then placed in 1 ml of buffer, consisting of 0.2 mM-pyridoxal 5-phosphate (P-5-P), 4 mM-dithiothreitol (DTT), 1 mM-EDTA and 50 mM-sodium phosphate, and homogenized using a Brinkman Polytron at level 3 for 40 s. The material was then sonicated for 25 s at setting 1, then centrifuged (3000 *g*) for 1 h in a Microfuge. The clear supernatant was removed and stored at -70°C until the assay was performed. ODC activity in the clear supernatant was determined by measuring the release of $^{14}\text{CO}_2$ from DL-[^{14}C]ornithine as previously described (Verma *et al.*, 1979). The final assay mixture contained 50 mM-Tris/HCl, 1.0 mM-EDTA, 0.1 mM-pyridoxal phosphate, 15 mM-DTT, 0.4 mM-L-ornithine and $0.4 \mu\text{Ci}$ of DL-[^{14}C]ornithine, including $188 \mu\text{l}$ of the supernatant in a final volume of $200 \mu\text{l}$. The reaction was carried out in 15 ml snap-top tubes (Falcon) in which a paper disc (Schleicher and Schuell), soaked in toluene and NCS scintillant (Amersham), was suspended on a 22-gauge needle. Each sample was incubated at 37°C for 60 min, with the released $^{14}\text{CO}_2$ being absorbed on to 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 $^{14}\text{CO}_2$. The discs were then transferred to vials containing 10 ml of Omnifluor (du Pont). Radioactivity was measured in a Beckman liquid-scintillation counter. Assays were always carried out in triplicate. Protein content was determined using the Bradford (1976) assay, with BSA as the standard.

RNA analysis

After the mice had been killed, the dorsal skin was removed and homogenized in 9 ml of guanidine thiocyanate solution at 4°C . Total cellular RNA was isolated as previously described (Chirgwin *et al.*, 1979). RNA was size-fractionated by formaldehyde/agarose-gel electrophoresis, stained with ethidium bromide to assess the integrity and migration of the RNA, and transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH, U.S.A.). The RNA was fixed to the membrane by u.v.r. cDNA probes for mouse ODC and chicken actin were labelled by the random-priming technique to a specific radioactivity of approx. $5 \times 10^8 \text{ c.p.m./}\mu\text{g}$ (Feinberg & Vogelstein, 1983). After overnight prehybridization in $1 \times$ Denhardt's/4 $\times$ SSC/salmon sperm DNA (200 $\mu\text{g/ml}$)/deionized 40% (v/v) formamide/5% (w/v) dextran sulphate/0.014 M-Tris, pH 7.4, hybridization was performed in the same solution with $1 \times 10^6 \text{ c.p.m./ml}$ of ^{32}P -labelled cDNA probe for 24 h at 42°C . Final washing conditions were $0.1 \times \text{SSC}/0.1\%$

SDS at 50°C . Autoradiography was performed using Kodak X-Omat film at -70°C . Autoradiograms were quantified by scanning with a laser densitometer.

RESULTS

Groups of mice (three mice per group) were exposed to increasing doses of u.v.B (45, 90, 180, 270 mJ/cm^2). A control group was not exposed to u.v.B, but was otherwise treated identically. At 24 h after irradiation the mice were killed and epidermal ODC activity was determined. ODC activity was increased above control values by all doses of u.v.B tested, with a peak increase (15.6-fold greater than control) detected after exposure to 90 mJ/cm^2 (see Fig. 1). To determine the time course of the u.v.B induction of ODC activity, mice were exposed to 90 mJ/cm^2 and killed at multiple time points. A minimum of three mice were studied at each time point. The results of this experiment are shown in Fig. 2. No significant change in ODC activity was detected either 30 or 60 min after irradiation. At 2 h a 7-fold increase in ODC activity was observed, with significant

Fig. 1. Effect of increasing doses of u.v.B on ODC activity in murine epidermis *in vivo*.

Skh hairless mice were sham-irradiated (C) or irradiated with u.v.B (45, 90, 180 or 270 mJ/cm^2) and killed at 24 h. Epidermal ODC activity was measured and is reported as $\text{nmol of CO}_2/\text{h per mg of protein}$. The sham-irradiated control was measured at $1.26 \text{ nmol of CO}_2/\text{h per mg of protein}$. Three mice were used at each dosage, and individual ODC values for each mouse were determined in triplicate. The standard errors of the ODC determinations were consistently less than 10%.

Fig. 2. Time course of u.v.B induction of ODC activity in murine epidermis *in vivo*.

Mice were exposed to 90 mJ/cm^2 of UVB. At 0.5 (30 min), 1, 2, 6, 12, and 24 h after irradiation, groups of three mice were killed; epidermal ODC activity for each mouse was measured in triplicate, and is reported as $\text{nmol of CO}_2/\text{h per mg of protein}$. The sham-irradiated control (C) ODC activity was $0.59 \text{ nmol of CO}_2/\text{h per mg of protein}$.

Fig. 3. Northern-blot analysis of murine epidermal RNA after exposure to increasing doses of u.v.B

Total cellular RNA was extracted from murine skin 24 h after exposure to 0 (control, C), 45, 90, 180 and 270 mJ/cm² of u.v.B and subjected to Northern-blot analysis. Each lane contained 10 µg of total cellular RNA. Blots were hybridized with cDNA probes for ornithine decarboxylase (ODC) and actin (A).

induction (32-fold increase over control at 12 h) observed from 12 to 24 h after irradiation. Previous studies have shown a good correlation between ODC enzyme activity and the amount of enzyme present as quantified by radioimmunoassay (Gilmour *et al.*, 1985; Verma *et al.*, 1986).

To determine whether the u.v.B-induced increase in ODC enzyme activity was associated with an increase in ODC-gene expression, total cellular RNA was isolated from both control and irradiated mice. Mice were exposed to the same doses of u.v.B as noted above and killed 24 h after irradiation. Northern-blot analysis of total cellular RNA prepared from mouse epidermis demonstrated the presence of two distinct ODC mRNA transcripts, approx. 2.2 and 2.7 kb in size (Fig. 3a). The presence of two ODC mRNA transcripts has been demonstrated in a variety of tissues, including neonatal rat keratinocytes (Rosen *et al.*, 1990), and has been shown to be due to the differences in the 3'-untranslated region of the ODC mRNA transcript (Berger *et al.*, 1984; Gilmour *et al.*, 1985; Hickok *et al.*, 1986; Kanamoto *et al.*, 1987). An increase in the amounts of both transcripts was noted with all doses of u.v.B studied, with a maximum increase (approx. 5-fold over control as assessed by laser densitometry) seen after exposure to 90 mJ/cm² (Fig. 3a). With greater doses of u.v.B, less marked induction of ODC mRNA transcripts was detected. The observation that ODC mRNA transcripts are maximally induced at 90 mJ/cm², and then decline, is in good agreement with the results obtained by measuring ODC activity at identical doses of u.v.B (Fig. 1). To determine the relative specificity of the u.v.B-induced increase in ODC-gene expression, the Northern blot shown in Fig. 3(a) was rehybridized with a cDNA probe for actin, a constitutively expressed 'housekeeping' gene. No significant increase in actin mRNA was detected (Fig. 3). The results of these experiments suggested that the u.v.B-induced increase in ODC enzyme activity was attributable, at least in part, to an increase in ODC-gene expression.

To determine the time course of the u.v.B-induced increase in

Fig. 4. Northern-blot analysis of the temporal UVB-induction of ODC gene expression

Mice were either sham-irradiated (C) or exposed to 90 mJ/cm² of u.v.B. Groups of three mice were killed at 0.5 (30 min), 1, 2 and 6 h. Total cellular RNA (10 µg/lane) was subjected to Northern-blot analysis. The blot was hybridized with cDNA probes for ODC and actin (A).

Fig. 5. Northern blot analysis of the effect of cycloheximide (Cyclo) on u.v.B induction of ODC mRNA transcripts

A group of mice was treated with intraperitoneal cycloheximide (70 mg/kg) and a second control group (Control) was not. At 4 h afterwards half of the mice in each group were exposed to 90 mJ/cm² of u.v.B ('U.v.'). After 24 h the mice were killed, total cellular RNA was prepared, and Northern-blot analysis was carried out using cDNA probes for ODC and actin (A). N.I., not irradiated.

ODC mRNA levels, mice were irradiated with 90 mJ/cm² of u.v.B, and killed at multiple time points after exposure. Northern-blot analysis (Fig. 4) demonstrated that an increase in ODC mRNA transcripts was detected by 2 h, with maximal induction noted at 6 h. No further induction of ODC mRNA transcripts was noted at 12 or 24 h (results not shown). These results are in good agreement with the temporal induction of ODC enzyme activity. No significant change in the amount of actin mRNA transcripts was noted after rehybridization of the same Northern blot with an actin cDNA probe.

To study the importance of new protein synthesis for the u.v.B induction of ODC gene expression, mice were treated with cycloheximide, a known protein-synthesis inhibitor. One group of mice received intraperitoneal cycloheximide (70 mg/kg) (Verma *et al.*, 1979) and a control group did not. After 4 h, mice from each group were either exposed to 90 mJ/cm² of u.v.B or sham-irradiated. After 24 h the mice were killed, and total cellular RNA was isolated from dorsal skin. Northern-blot analysis of total cellular RNA was carried out, and the blot was hybridized with cDNA probes for ODC and actin. The results of this experiment are shown in Fig. 5. Cycloheximide treatment did not alter the basal levels of ODC mRNA transcripts in control sham-irradiated mouse skin, nor did it inhibit the u.v.B-induction of ODC mRNA transcripts. No significant effect of cycloheximide on actin mRNA transcript levels was noted.

DISCUSSION

U.v.r. is an environmental carcinogen, causing cutaneous neoplasia in both mouse and man. However, the molecular mechanisms underlying u.v.B action remain poorly understood. Our data demonstrate that the u.v.B induction of ODC activity in the hairless mouse is due in part to induction of ODC-gene expression. These results are in good agreement with studies carried out using newborn-rat keratinocytes cultured *in vitro* (Rosen *et al.*, 1990), and extend the validity of this experimental paradigm to the *in vivo* setting.

The mechanisms responsible for increased expression of the ODC gene are complex and have been shown to include changes in gene transcription (Kontula *et al.*, 1984; Katz & Kahana, 1987) as well as changes in mRNA stability (Rose-John *et al.*, 1987; Hölttä *et al.*, 1988). Discrepancies between changes in the relative levels of ODC mRNA transcripts and enzyme activity have been noted previously, raising the possibility that significant regulation of ODC enzyme activity may take place at, or distal to, the level of translation (Chang & Chen, 1988; Hölttä *et al.*, 1988). Further support for the importance of post-transcriptional and in fact post-translational regulation derives from an elegant series of experiments which demonstrated that information required for ODC mRNA induction resides within the ODC protein-coding sequence and not the 5'-flanking region of the ODC gene (van Daalen Wetters *et al.*, 1989a,b).

The potential mechanisms responsible for the u.v.B-induction of ODC-gene expression include an increase in ODC-gene transcription and/or a change in ODC mRNA stability. Although u.v.B has been noted to increase the expression of a number of different genes, including interleukin-1 (Kupper *et al.*, 1987), hsp 70 (Brunet & Giacomoni, 1989), and c-H-ras and c-myc (Ronai *et al.*, 1988), the specific pathway(s) for u.v.B induction of gene expression have not yet been defined. For example, no u.v.B-response element has been characterized in the 5'-flanking regions of u.v.B-inducible genes. The finding that u.v.B irradiation of both mouse epidermis and rat keratinocytes leads to a rapid, reproducible and specific induction of ODC-

gene expression raises the possibility that u.v.B may exert its effects via an increase in gene transcription. It is intriguing to note that the relative induction of ODC activity was much greater (32-fold maximal induction of ODC activity versus 5-fold induction of ODC mRNA transcripts) than the u.v.B induction of ODC-gene expression, suggesting that most of the u.v.B induction of ODC activity may take place at a post-transcriptional level. The results of these studies suggest that the u.v.B induction of ODC activity in the Skh hairless mouse may be a useful model for further analysis of the molecular effects of u.v.B radiation.

This work was supported in part by grants from the Medical Research Council of Canada, NCI (National Cancer Institute) Canada and the Cancer Research Society. D. J. D. is a Career Scientist of the Ontario Ministry of Health.

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