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Photoaugmentation in the Hairless Mouse: A Study Using Ornithine Decarboxylase Activity and Alteration of DNA Synthesis as Markers of Epidermal Response

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Photoaugmentation is the potentiation of UVB-induced cutaneous erythema by UV irradiation. We have examined other cutaneous responses to UVB irradiation-the 4 hr depression of DNA synthesis, the 48 hr stimulation of DNA synthesis, and the induction of ornithine decarboxylase (ODC), to determine whether these were also susceptible to augmentation by UVA, which does not cause these responses when administered alone.

No photoaugmentation of DNA synthesis, stimulation or ODC induction occurred. The early depression of DNA synthesis was slightly augmented but this did not consistently reach significance.

While investigating possible protective effects of UVA-induced immediate pigment darkening upon UVB-induced erythema in humans, Willis, Kligman, and Epstein [1] found that UVA radiation, given before or after UVB, greatly enhanced the erythema response. This phenomenon was believed to signify a sensitization of the skin by longwave UV irradiation to the effects of UVB, and was described as photoaugmentation. The effect has recently been confirmed by Boer, Schothorst, and Suurmond [2]. Other workers [3] have demonstrated that the human erythema response to different UV wavelengths in threshold dose ranges is linearly additive rather than augmentative, and referred to the cumulative effect of different wavelengths as photoaddition. Van der Leun and Stoop [4] found that UVB erythema was diminished by subsequent exposure to sunlight filtered through window glass to remove UVB. There is thus some disagreement as to whether the response of the skin to different wavelengths is augmentative, additive, or more complex.

The study of biological responses other than erythema could help to clarify possible interactions between different types of UV light. In a recent study, Kaidbey, Grove, and Kligman [5] were unable to demonstrate photoaugmentation of UVB-induced sunburn cell production by longwave UV light. Examining edema of the mouse tail induced by UVB irradiation, significant photoaugmentation by UVA at dosages of up to 58 J/cm^2 could not be demonstrated by Bjellerup and Moller [6] although the phototoxic edema response induced by UVA in drug-photosensitized animals was increased by the addition of UVB.

We have measured 2 responses of the skin to UVB, the alteration of DNA synthesis, and the induction of ornithine decarboxylase (ODC), looking for alteration by addition of UVA irradiation. Neither response occurs following UVA irradiation alone. Early depression of scheduled DNA synthesis probably reflects damage to nuclear material, and is seen at 2–4 hr. A phase of increased epidermal proliferation is seen at 48–72 hr and may reflect repair to damaged epidermal cells and the thickening response to UVB irradiation [7]. The significance of ODC induction by UV light is not fully understood: ODC is the rate-limiting enzyme in polyamine biosynthesis [8], and is found in increased amounts in proliferating tissues. High levels of ODC activity in the skin are consistently induced by the application of tumor-promoting chemicals [9] and the shorter wavelengths of UV light [10]. The induction of ODC by UVB is biphasic, with 5–6 hr and 24–28 hr peaks; it has been suggested that these may be related to transcriptional and translational activity respectively [11].

In this study we have evaluated possible photoaugmentation by UVA of the 4 hr depression and 48 hr stimulation of DNA synthesis, and the 6 and 24 hr peaks of ODC activity, induced following a single irradiation with UVB.

MATERIALS AND METHODS

Animals

Two- to 4-mo old female hairless albino mice were used for all experiments. Animals were irradiated in individual compartments (3 × 8 × 3 cm) providing restraint and even irradiation of dorsal skin.

Light Sources

UVA: Four F40 BLB lifetime fluorescent tubes (Sylvania) were mounted 12.5 cm apart in a foil-lined holder; animals were irradiated at a distance of 55 cm. This provided a dose of 0.12 J/cm^2/min measured using an LM 301 UVA meter (National Biological Corporation).

UVB: Animals were irradiated with four FS40 sunlamp bulbs (Westinghouse) mounted in a holder, at a distance of 65 cm, providing a dosage of 3.6 mJ/cm^2/min, measured using an IL500 Research Radiometer equipped with a UVB probe.

Experimental Design

To evaluate photoaugmentation of UVB-induced alteration of DNA synthesis, we compared 3 groups of animals in each experiment. Group I received UVB alone. Group II received UVB at the same dosage, followed by UVA. Group III received UVB alone at a dose 2-fold greater than Groups I and II. In this way, we could (a) demonstrate that the effect of UVB in Groups I and II was not maximal, and therefore might potentially be augmented by UVA in Group II and, (b) compare the effect, if any, of UVA in Group II with the effect of a twofold increase in UVB dosage in Group III.

For ODC studies a similar design was employed except that a 1.5-fold increase in Group III was employed, since this was sufficient to induce significantly greater ODC activity. UVA dosages similar to those used in other photoaugmentation studies were employed [1, 5]. UVA:UVB energy ratios were between 780:1 and 3125:1. Since the UVA:UVB spectral irradiance ratio in sunlight varies between 110 and 1650 according to the solar zenith angle, and since UVA is between 200 and 2000 times less erythemogenically effective than UVB [12], ratios of this size are appropriate for study.

DNA Synthesis Assay

Animals received 25 μCi ^{3}H-TdR (specific activity 1 Ci/mM) by intraperitoneal injection. One hour later they were sacrificed and the skins removed and spread on paper towels. TdR incorporation into epidermis was measured by the method of Otani et al [13–15]. In brief, fixed areas of epidermis were isolated from dorsal and ventral sites as follows: After heating the skin at 56°C for 10 min, 17-mm plastic discs were glued to the skin using cyanoacrylate adhesive (Krazy glue), and the discs and underlying skin cut out and placed in scintillation vials

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Abbreviations:
ODC: ornithine decarboxylase

containing 2 M potassium bromide. The vials were heated at 56°C causing the dermis to separate. The disc and adherent epidermis were incubated with 0.25% acetic acid and distilled water to remove unincorporated ^{3}H-TdR, and dehydrated with 95% ethanol. Scintillation fluid (PCS II, Amersham) was then added to the vial which was counted in a liquid scintillation counter. Counts in dorsal epidermis were expressed as a percentage of the counts in ventral (nonirradiated control) epidermis. Previous experiments showed no significant difference in counts in the two sites in untreated animals.

Ornithine Decarboxylase Assay

Enzyme activity was determined by measuring the release of $^{14}CO_2$ from DL-(1-^{14}C)-ornithine by the method of Russell and Snyder [8] with minor modifications.

Enzyme Preparation: Animals were sacrificed by neck dislocation; dorsal skin was removed immediately. Epidermis was separated by immersion of the skin in 50 mM phosphate buffer at 56°C for 15 seconds, followed by immediate cooling in buffer at 0°C; the skin was then spread on paper, epidermis upward. The epidermis was removed by gentle scraping and placed in preweighed tubes surrounded by ice. In each experiment individual skins were assayed; results are the mean of 6 to 8 animals. The epidermis was homogenized for 15 seconds in 19 volumes of 50 mM phosphate buffer containing 0.2 mM pyridoxal phosphate, 1 mM EDTA, and 4 mM dithiothreitol, using a Brinkman homogenizer. The homogenate was centrifuged at 20,000 g for 10 min at 0°C, giving a clear supernatant.

Ornithine substrate: 1.2 mM ornithine hydrochloride was dissolved in buffer containing pyridoxal phosphate, EDTA, and dithiothreitol as above. 0.5 μCl DL-(1-^{14}C)-ornithine hydrochloride/0.1 ml substrate was added.

Procedure: 0.2 ml of enzyme preparation was put in each 15 ml tube. 0.1 ml of labeled substrate was added, and the tube closed with a rubber stopper fitted with a center well assembly (Kontes) containing a filter paper bearing 0.05 ml NCS tissue solubilizer (Amersham), for CO_2 absorption. The tubes were incubated for 60 min at 37°C in a shaking water bath. The reaction was terminated by the addition of 0.3 ml 2M citric acid. Incubation was continued for 1 hr to ensure complete absorption for $^{14}CO_2$ by the NCS. Filter papers were then transferred to scintillation vials containing 5 ml of OCS (Amersham). Blank tubes contained no enzyme. Release of $^{14}CO_2$ was linear up to 1 hour and was linearly related to concentration of epidermal extract.

RESULTS

DNA Synthesis

In nonirradiated animals DNA synthesis as measured by epidermal TdR incorporation is comparable in dorsal and ventral skin samples (dorsal/ventral cpm 1 hr after ^{3}H-TdR 102 ± 10% n = 12).

The effects of irradiation are shown in Table I; each experimental group contained ten animals. In each experiment DNA synthesis in dorsal irradiated skin, expressed as a percentage of the values for ventral nonirradiated skin, was depressed 4 hr after UVB irradiation (Group I). The significantly greater depression resulting from a 2-fold increase in the dose of UVB (Group III) confirmed that the depression in Group I was not maximal.

The effect of additional UVA irradiation in Group II is less clear. In two experiments, A and B, in which mice in Groups I and II received 7.2 mJ/cm^2 UVB, additional UVA in Group II resulted in slightly greater depression but this did not reach significance. In a third experiment (C) in which a larger dose of UVB was employed (14.4 mJ/cm^2 to Groups I and II) greater depression again occurred in Group II which received UVA in addition; this was significantly greater than Group I and was of the same magnitude as the depression induced by 28.8 mJ/cm^2 UVB alone (Group III).

Forty-eight hours after irradiation, increased thymidine incorporation occurred (Table III); this response was not increased by UVA irradiation.

ODC Activity

ODC activity in Groups I and II did not differ significantly (Table II). Irradiation with UVA in addition to UVB did not therefore increase ODC induction. In contrast, irradiation with 1.5 × greater UVB dosage without UVA caused significantly greater ODC induction at both time points (6 hr, $p \approx 0.05$, 24 hr $p < 0.05$). Irradiation with UVA alone causes no induction of ODC at doses of up to 40 J/cm^2.

DISCUSSION

Interactions between the effects of different wavelengths of UV light upon the skin are poorly understood. In defining safety standards for human UV exposure, additive effects of different wavelengths are assumed [16], and a linearly additive effect of different wavelengths at threshold dose ranges does seem to hold for the erythema response in humans [3, 17]. The contribution of longwave UV to other biological responses of the skin has not been widely studied. UVB has clearly defined effects upon the skin other than erythema, including components of inflammation such as edema, leukocyte infiltration, cell damage with sunburn cell formation, as well as reparative and protective responses such as epidermal thickening and delayed tanning. UVB is also believed to be responsible for the carcinogenic effects of sunlight, and the connective tissue changes associated with aging. Traditionally, long wavelength UV has been considered harmless as far as aging, carcinogenesis and sunburn are concerned. However, experimental data on interactions between long and medium wavelengths is limited. The erythema response has been most studied in this respect, and it does appear that suberythema doses of UVA and of UVB in combination may cause erythema [17]. This has been considered by some workers to be an additive effect [3], but the histological changes in the 2 types of erythema are different [18], so the interaction may be more complex. Willis, Kligman and Epstein [1] referred to the potentiation of UVB erythema by UVA as photoaugmentation, with longwave radiation making the skin more responsive to UVB. Since both types of radiation can cause erythema when given alone, the question is hard to resolve. Kaidbey, Grover, and Kligman [5], examining sunburn cell formation, which does not occur significantly following UVA irradiation alone [19], was not able to "photoaugment" UVB induced sunburn cell formation in mouse skin by irradiation with UVA in addition. Photoaugmentation by UVA or UVB-induced skin cancer formation in mice has not been demonstrated [12] provided that carefully filtered UVA sources have been employed, to ensure that additional UVB was not administered, when UVA was given intermittently. We have evaluated other responses of the skin which occur following UVB irradiation but not after UVA. No increase in UVB-induced epidermal ODC activity was seen if animals received UVA irradiation in addition, when either the early (6 hr) or late (24 hr) peaks of activity were examined. In contrast, a 1.5-fold increase of UVB dose did induce greater ODC activity, confirming that we were examining a submaximal and therefore potentially augmentable response.

Examining epidermal thymidine incorporation 48 hr after irradiation, a similar result was obtained. Mice in Groups I and II showed a similar degree of increase in dorsal epidermal thymidine incorporation and did not differ significantly from each other; contrasting with the markedly increased proliferative response to a larger dose of UVB.

The depression of DNA synthesis 4 hr after UVB irradiation was inconsistently increased by the addition of UVA (Table I). In each of these experiments, UVA irradiation was followed by a greater degree of depression but in only one experiment was this significant at the 95% level. In this experiment (C), the effect of UVA was similar to the effect of a 2-fold increase in UVB dosage (Group III). In experiments A and B the effect of UVA was less than the effect of a 2-fold increase in UVB. While it is possible that UVA may increase the depression of DNA synthesis induced by UVB, our technique is insufficiently sensitive to demonstrate it consistently. At 48 hr, no augmentation by UVA of the proliferative response to UVB was observed.

TABLE I. *Four hour ^3H thymidine incorporation in dorsal (irradiated) epidermis expressed as a percentage of incorporation in ventral (control) epidermis*

	Group I UVB Only		Group II UVB + UVA				Group III UVB Only		
Expt.	UVB dose mJ/cm^2	D/V %	UVB dose mJ/cm^2	UVA dose J/cm^2	D/V %	p Group I vs. Group III	UVB dose mJ/cm^2	D/V %	p Group III vs. Group 1
A	7.2	57 ± 5.0	7.2	22.5	44 ± 4.9	$p < 0.1$	14.4	37 ± 4.7	$p < 0.01$
B	7.2	86 ± 7.4	7.2	22.5	76 ± 6.3	$p < 0.3$	14.4	63 ± 5.8	$p < 0.02$
C	14.4	47 ± 5.7	14.4	22.5	29 ± 2.7	$p < 0.01$	28.8	30 ± 4.1	$p < 0.02$

TABLE II. *Forty-eight hour ^3H thymidine incorporation in dorsal (irradiated) epidermis expressed as a percentage of incorporation in ventral (control) epidermis*

Group I UVB only		Group II UVB + UVA				Group III UVB only		
UVB Dose mJ/cm^2	D/V %	UVB Dose mJ/cm^2	UVA Dose J/cm^2	D/V %	p Group I vs. Group II	UVB dose mJ/cm^2	D/V %	p Group III vs. Group 1
14.4	185 ± 25.8	14.4	22.5	133 ± 14.2	N.S.	28.8	484 ± 44.8	$p < 0.001$

TABLE III. *ODC activities in groups irradiated with UVB or UVB + UVA Sacrificed 6 and 24 hr after irradiation*

	Group I UVB only		Group II UVB + UVA			Group III UVB only	
	UVB Dose mJ/cm^2	ODC Activity nM CO_2/10 mg tissue/hr	UVB dose mJ/cm^2	UVA dose J/cm^2	ODC Activity nM CO_2/10 mg tissue/hr	UVB dose mJ/cm^2	ODC activity nm CO_2/10 mg tissue/hr
6 Hour 8/Group	28.8	0.307 ± 0.058	28.8	22.5	0.264 ± 0.033 I vs. II N.S.	43.2	0.482 ± 0.064 I vs. III $p \approx 0.05$
24 Hour 6/Group	18	0.486 ± 0.097	18	22.5	0.481 ± 0.084 I vs. II N.S.	27	1.116 ± 0.263 I vs. III $p < 0.05$

Animals receiving UVA showed slightly lower levels of thymidine incorporation in dorsal epidermis but this was not significant. We were therefore not able to demonstrate a consistent effect of UVA irradiation either upon the depression of thymidine incorporation which results in damage to DNA induced by irradiation, or upon the proliferative response which manifests as epidermal thickening and scaling. Similarly no effect was demonstrable upon either of the peaks of ODC activity induced by UVB. If photoaugmentation exists, it may be confined to the erythema response; alternatively, "photoaddition" is occurring: since sunburn cell formation, tumor induction, ODC induction, and the early depression of DNA synthesis and later stimulation of DNA synthesis do not occur following longwave radiation alone, no increase as a result of addition of UVA to UVB would be predicted. Additive effects could also account for the increased edema response to chemical UVA mediated phototoxic reactions brought about by the addition of UVB irradiation [6].

While the ability of compounds to induce ODC has been proposed as a marker for the tumor-promoting ability [20], recent studies indicate that this relationship is by no means specific or universal [21, 22] and that the ability to induce ODC may better reflect the degree of epidermal hyperplasia induced. Furthermore, it is not possible to extrapolate from chemical tumor promotion to tumor induction by UV light. However, the lack of photoaugmentation of ODC induction would be consistent with tumor studies in mice which have shown that intermittent UVA irradiation is not carcinogenic, and that UVA added to UVB in the ratio that is present in noontime sunlight does not augment or add to the carcinogenic effects of UVB [12].

Measurements of cutaneous reactions to light have in the past largely hinged upon the evaluation of erythema, edema or histological damage. The measurement of biochemical responses provided an alternative approach to the evaluation of interactions between different wavelengths of light, skin and photosensitizing agents.

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