

# Inhibition of Ultraviolet-B Epidermal Ornithine Decarboxylase Induction and Skin Carcinogenesis in Hairless Mice by Topical Indomethacin and Triamcinolone Acetonide<sup>1</sup>

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## ABSTRACT

Modulation of ultraviolet-B (UVB) skin carcinogenesis by topical treatment with two antiinflammatory drugs expected to have different mechanisms of action has been studied in the hairless mouse. Indomethacin is a nonsteroidal antiinflammatory agent which may act by inhibiting prostaglandin biosynthesis. Triamcinolone acetonide is a steroidal antiinflammatory agent. Both of these drugs inhibited the induction of epidermal ornithine decarboxylase by UVB when applied topically in an acetone vehicle. A UVB skin tumor study was designed. Groups of mice were irradiated daily with UVB for 20 days, each mouse receiving a total of 17.1 kJ UVB per sq m. Group 1 was treated with acetone immediately after each irradiation; Group 2 received 700 nmol indomethacin in acetone immediately after each irradiation; Group 3 received 14.4 nmol triamcinolone acetonide in acetone immediately after each irradiation. Mice were killed after 52 weeks, and the tumors were excised and examined histologically. Both topical indomethacin and topical triamcinolone acetonide were effective in reducing the incidence and size of the skin tumors induced by UVB. This evidence supports the hypothesis that the induction of ornithine decarboxylase may be a critical component of UVB skin carcinogenesis and that inhibition of ornithine decarboxylase induction can be used as a screen for agents which will inhibit UVB skin carcinogenesis.

## INTRODUCTION

Induction of the polyamine-biosynthetic enzyme ODC<sup>3</sup> (EC 4.1.1.17) is an early event associated with tumor promotion. Cutaneous ODC is induced by a wide range of tumor promoters, including both chemical (8) and physical (2) stimuli, and also by UV (6). O'Brien (7) has proposed that ODC induction is an obligatory process in mouse skin carcinogenesis. Consequently, Verma *et al.* (12) suggested that the ability to inhibit OC induction by chemical tumor promoters could be used to assay for anti-tumor-promoting agents. We have been interested in extending these observations using carcinogenic wavelengths of UV radiation. Specifically, we wished to demonstrate the effectiveness of measuring the ability of different agents to inhibit the induction of epidermal ODC as an assay for potential inhibitors of UVB skin carcinogenesis.

We have reported previously (5) that single topical applications of the antiinflammatory drugs triamcinolone acetonide or indomethacin applied immediately after irradiation significantly inhibit the induction of ODC activity by UVB in hairless mouse epidermis. Triamcinolone acetonide also significantly inhibited UVB-induced epidermal DNA synthesis as measured by [<sup>3</sup>H] thymidine incorporation, but this was not the case for indomethacin, which showed no significant effect.

To test the relative effectiveness of these drugs as anticancer agents, we designed a tumor study in hairless mice. We discovered early in the study that chronic topical application of indomethacin to hairless mouse skin was toxic. The results of the toxicity study and the effects of indomethacin and triamcinolone acetonide on UVB carcinogenesis are presented in this report.

## MATERIALS AND METHODS

### Mice

Albino female SKH/Hrl hairless mice, 4 to 6 weeks old at the start of the study, were used throughout.

### Chemicals

L-Ornithine was obtained from Sigma Chemical Co. (St. Louis, Mo.). L-[1-<sup>14</sup>C]Ornithine hydrochloride (specific activity, 50 mCi/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). Indomethacin (Indocin) was obtained from Merck Sharp and Dohme (West Point, Pa.). Triamcinolone acetonide was a gift from Westwood Pharmaceuticals (Buffalo, N. Y.).

### UV Source

Irradiation was with Westinghouse FS40 sunlamps with a peak irradiance at 313 nm calibrated using an IL NBS 313 filter with an Optronics 751 V spectral radiometer. The radiation from the lamps was filtered through cellulose triacetate to remove contaminant UVC.

### Irradiation of Mice

Mice were irradiated with UVB from the cellulose triacetate-filtered FS40 sunlamps mounted 16 cm above the mouse dorsal skin. For the tumor studies, mice were irradiated 5 times per week for 4 weeks with gradually increasing amounts of UVB, rising from 1 MED (0.3 kJ/sq m) on the first day to 2 MED (0.6 kJ/sq m) on the second day and 3 MED (0.90 kJ/sq m) on all subsequent days. The amount of UVB administered was increased gradually to reduce the degree of ulceration and burning which can occur when hairless mice are irradiated with 3 MED daily without an initial acclimatization. Irradiation time was about 0.65 min for 1 MED, and UVB was delivered at a fluence rate of 7.7 J/sq m/sec. Each mouse received a cumulative dose of 17.1 kJ UVB per sq m.

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<sup>3</sup> The abbreviations used are: ODC, ornithine decarboxylase; UVB, ultraviolet-B (290 to 320 nm); UVC, ultraviolet-C (250 to 290 nm); MED, minimal erythema dose.

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### Toxicity Study

Groups of mice were painted on the dorsal skin with 0.70, 1.40, or 3.49  $\mu\text{mol}$  indomethacin in 0.1 ml acetone in a single application. The treatment was repeated daily for a maximum of 20 days, which is the length of time we had chosen for the UVB tumor studies. In a similar study, mice were painted once daily with 115 nmol triamcinolone acetonide for 20 days and observed for signs of any toxic reactions.

### Tumor Study

Mice (30/group) were irradiated daily for 20 days with UVB as described above. Immediately after each exposure to UVB, groups of mice were painted dorsally with (a) 0.1 ml acetone, (b) 700 nmol indomethacin in 0.1 ml acetone, or (c) 14.4 nmol triamcinolone acetonide in 0.1 ml acetone. Unirradiated control mice received 0.1 ml acetone only.

After 52 weeks, the experiment was ended, and all the mice were subjected to a thorough inspection. Probable tumors were excised for histological examination to determine the degree of cancer.

### Histology

Tumors were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin.

On histological examination, 3 types of lesions were observed in increasing order of malignant potential.

**Squamous Papillomas.** In these lesions, there were acanthosis of the epidermis, orthokeratotic hyperkeratosis of the stratum corneum, and a varying degree of papillomatosis. No atypical keratinocytes were seen.

**Atypical Keratoses (Actinic Keratoses and Digitate Keratoses).** These lesions showed atypical keratinocytic hyperplasia present most prominently in the lower one-half of the epidermis. The epidermis was acanthotic or atrophic, and the keratinocytes extended into the papillary dermis in "bud-like" fashion. The overlying stratum corneum showed confluent parakeratosis. The degree of papillomatosis present was variable (being more prominent in the digitate keratoses). The atypical keratinocytes contained pleomorphic nuclei, many of which were hyperchromatic or pyknotic. Occasional mitotic figures were seen.

**Squamous Cell Carcinomas.** In these lesions, there was a neoplasm composed of nests and islands of atypical keratinocytes present within the reticular dermis. The atypical keratinocytes showed marked nuclear pleomorphism and abundant eosinophilic cytoplasm with scattered intercellular bridges between the cells. Numerous mitoses were present, and scattered dyskeratotic cells showing individual cell keratinization were seen. The overlying stratum corneum was hyperkeratotic and contained parakeratosis.

### Assay of ODC

ODC induced by a single irradiation with 0.9 kJ UVB per sq m was assayed in epidermal extracts as described previously (5, 6).

## RESULTS

**Toxicity of Indomethacin and Triamcinolone Acetonide.** Topical administration of 0.70  $\mu\text{mol}$  indomethacin daily for 20 days to the mice produced no discernible signs of toxicity. Daily treatment with 1.40 or 3.49  $\mu\text{mol}$  indomethacin, however, was lethal, and the median survival times were 5 and 2 days, respectively. The mice apparently died of severe gastrointestinal bleeding.

Daily topical administration of 115 nmol triamcinolone acetonide for 20 days was nonlethal, but weight loss and lethargy were evident in some mice.

**Tumor Study.** The incidence of tumors, the mean tumor diameters, and the tumor types found in the various groups are summarized in Table 1. No tumors were present in mice which received vehicle only. The incidence of UVB-induced skin tumors was markedly reduced in the group treated with triamcinolone acetonide to one-half that of the acetone only treated, irradiated group. The tumors grew more slowly, having a mean diameter of 1.25 mm compared to 9.9 mm at 52 weeks (Table 1).

The indomethacin-treated group also showed a reduced tumor incidence, although this was not as marked as in the triamcinolone acetonide-treated group, to about three-fourths that of the acetone only treated, irradiated controls. Again, the mean tumor diameters were significantly decreased in the indomethacin-treated group compared to the controls (Table 1).

**Epidermal ODC Activity.** The tumor studies reported here were performed using an acetone vehicle rather than the 30% propylene glycol-70% isopropyl alcohol vehicle used previously (5). The use of an acetone vehicle enhanced the inhibitory effects of indomethacin on the induction of epidermal ODC by a single irradiation with UVB, suggesting that there was an increased penetration of the drug. Topical administration of 700 nmol indomethacin in acetone resulted in a 60% inhibition of ODC induction by UVB (Table 2), compared to the 53% inhibition by 1.40  $\mu\text{mol}$  indomethacin found previously using the propylene glycol-isopropyl alcohol vehicle. Triamcinolone acetonide (14.4 nmol) in an acetone vehicle gave 80% inhibition of ODC induction by UVB (Table 2), which is similar to that

Table 1  
Modulation of UVB skin carcinogenesis by indomethacin and triamcinolone acetonide

	Survival <sup>a</sup>	% of surviving mice with tumors	Tumor diameters (mm)	No. of tumor type <sup>b</sup>			Total
				Squamous papilloma	Atypical keratosis	Squamous cell carcinoma	
UVB + acetone	27	30	9.9 $\pm$ 8.2 <sup>c</sup>	2	5	1	8
UVB + indomethacin (700 nmol)	26	23	2.6 $\pm$ 1.7 <sup>d</sup>	2	3	1	6
UVB + triamcinolone acetonide (14.4 nmol)	27	15	1.25 $\pm$ 0.5 <sup>d</sup>	0	3	1	4

<sup>a</sup> Each group originally contained 30 mice; survival values are the number of mice alive after 52 weeks.

<sup>b</sup> See "Materials and Methods" for description.

<sup>c</sup> Mean  $\pm$  S.D.

<sup>d</sup> Mean tumor diameter significantly lower than UVB + acetone controls ( $p < 0.05$  by Student's *t* test).

Table 2  
Comparison of relative tumor incidence and inhibition of ODC activity by indomethacin and triamcinolone acetonide

	% of tumor incidence <sup>a</sup>	ODC activity <sup>b</sup>	
		nmol/mg/hr	% of controls
UVB + acetone (control group)	100	1.91 ± 0.30 <sup>c</sup>	100
UVB + indomethacin	77	0.76 ± 0.24	40
UVB + triamcinolone acetonide	50	0.38 ± 0.18	20

<sup>a</sup> Number of surviving mice with tumors in each group expressed as a percentage of UVB + acetone control group.

<sup>b</sup> Epidermal ODC measured 24 hr after exposure to a single irradiance with 0.9 kJ/sq m.

<sup>c</sup> Mean ± S.D. of epidermal extracts from 5 mice.

reported previously in a propylene glycol-isopropyl alcohol vehicle (5).

## DISCUSSION

Inhibition of UVB skin carcinogenesis by 2 antiinflammatory agents with widely differing properties and which inhibit epidermal ODC induction adds further support to the hypothesis that, as may be the case with chemical carcinogenesis, induction of ODC is an essential component in UVB skin carcinogenesis.

The anticancer action of indomethacin is likely to be complex. Indomethacin inhibits prostaglandin synthesis in skin (4), and this may be important in both its antiinflammatory and anticarcinogenic effects. Verma *et al.* (11) have shown that indomethacin will inhibit phorbol ester skin tumor promotion and that this could be reversed by concurrent additions of prostaglandins E<sub>1</sub> or E<sub>2</sub>; these prostaglandins were not capable of inducing epidermal ODC alone nor of acting as skin tumor promoters. Indomethacin has been used clinically to successfully alleviate skin cancers in patients with xeroderma pigmentosum (1), a disease in which the photorepair mechanism responsible for repairing DNA damage caused by UV radiation is defective. Unfortunately, because of the toxicity of indomethacin in chronic doses, it was not possible to study its effects on UVB carcinogenesis at higher concentrations. Higher concentrations of indomethacin delivered acutely inhibited ODC induction to a greater extent than did lower concentrations, which may suggest that the anticarcinogenic action of indomethacin would have been more pronounced if the dose were higher.

The mechanism by which glucocorticoids inhibit mouse skin carcinogenesis may be related to their ability to inhibit DNA synthesis, thus counteracting the induction of hyperplasia (10). In support of this, topical triamcinolone acetonide was found to inhibit the increased epidermal DNA synthesis normally evident

48 hr after irradiation with UVB, whereas topical indomethacin had no significant effect (5).

The mechanism of UVB skin carcinogenesis is obscure. However, the complex cascade of events triggered by UVB in the skin, which apparently lead to neoplastic growth, includes several features also associated with the process of chemical carcinogenesis. UV radiation can act as an initiator in the 2-step initiation-promotion sequence (3, 9). In common with chemical tumor promoters (such as 12-O-tetradecanoylphorbol-13-acetate), UVB induces epidermal hyperplasia, ODC activity, and DNA synthesis. In the present study, it has been demonstrated that, as with chemical carcinogenesis, it is possible to inhibit UVB skin carcinogenesis using antiinflammatory drugs which will inhibit ODC induction. The results of this study support the usefulness of measuring inhibition of the induction of ODC in the epidermis as a rapid assay for potential inhibitors of UVB skin carcinogenesis.

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