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STUDIES ON THE INFLUENCE OF ULTRAVIOLET LIGHT ON INITIATION IN SKIN TUMORIGENESIS*

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ABSTRACT

The effect of shortwave ultraviolet (UV) light applied once or 10 times on initiation by 7,12-dimethylbenz(a)anthracene (DMBA) in two-stage skin carcinogenesis was studied. Croton oil was used as promoter. The results showed that a single UV treatment increased the formation of benign tumors when given prior to initiation-promotion. The incidence of benign tumors decreased significantly when 10 doses of UV light were given after initiation, although a few carcinomas and sarcomas occurred, suggesting a summative effect of both agents.

In 1944, Mottram proposed that carcinogens act in 3 successive stages in skin: as a sensitizing factor, as the cause of a specific cellular reaction leading to tumor cell formation, and as a developing factor causing the formation of a visible tumor [1, 2]. Berenblum and Shubik [3–5] showed that initial tumor cell formation (initiation) is unaffected by previous applications of certain chemicals, with the decisive factor being the posttreatment (promotion) with croton oil. In large doses, ultraviolet (UV) light is known to be an effective skin carcinogen [6] as well as an "initiator" [7]. In small doses, UV light produces mitotic stimulation, epidermal hyperplasia, and vascular and dermal alterations in skin [8]. The following experiments were performed in order to study the modifying effects of UV light on initiation of skin carcinogenesis.

MATERIALS AND METHODS

Female Swiss mice, 8 weeks old, were housed in plastic cages (10 per cage) and fed diet pellets and water ad libitum. The UV light source was a Westinghouse FS40T12 sunlamp emitting a total energy of 1.7×10^7 ergs/cm²/hr at a distance of 15 inches as measured with an International light IL 335 exposure meter. The UV treatment consisted either of one 3-hr treatment given once or 10 treatments given 5 times a week for 2 weeks for a total of 30 hr. As initiator 50 μg 7,12-dimethylbenz-(a)anthracene (DMBA) in acetone was applied once. As promoter 0.02 cc of a 2.5% solution of croton oil in reagent-grade acetone was applied 2 times a week for 30 weeks on the shaved skin between the flanks. The UV treatment was given 1 hr before or 1 hr after the DMBA treatment. Croton oil applications were started 7 days after the initiation.

The animals were checked weekly and tumors were recorded. The tumors were recorded when they reached 0.5 cm in diameter and had been present at least 3 weeks. All animals survived to 30 weeks at which time they were killed and complete autopsies performed. Sections from skin lesions as well as organs showing gross abnormalities were studied histologically. Formalin-fixed sections were embedded in paraffin and stained with hematoxylin/eosin, PAS, Masson, Kreyberg, Gomori, and Weigert's stains.

RESULTS

Details of treatment, number of animals per group, frequency of applications, dose, and the ensuing tumor response are shown in the Table.

In the control groups given DMBA once (Group A) and UV light once (5.1×10^7 ergs/cm²) (Group B) no tumor nor any significant permanent changes in the epidermis were seen. Ten applications of UV light (Group C) produced ulcerations and subsequent scarring of skin in many animals. Two squamous cell papillomas were seen in this group. Croton oil alone (Group D) produced slight hyperplasia of epidermis but no tumors, with hyperkeratosis being the predominant feature.

DMBA once followed by croton oil (Group E) produced a number of benign tumors in the skin, some of which regressed (Fig.). The first papilloma was found 4 weeks after beginning treatment. The papillomas were of different types; some were pedunculated with an acanthotic squamous epithelium. The majority were composed of proliferating, keratinizing epithelium around a stromal papilla. Some were composed of a thick protruding dermis with accumulations of collagen bundles covered by a thin epidermis. Two keratoacanthomas were also seen consisting of proliferating squamous epithelium on a cup-shaped base. The dermis showed accumulations of mast cells as well as thickened bundles of collagen fibers.

In the animals given UV light once followed by DMBA and then continuous croton oil treatment (Group F), ulcerations and scarring of skin were seen at the beginning. The tumors occurring later were mostly papillomas, similar to those seen in the previous group. Some of the tumors regressed and not all tumors grossly observed were available for histologic examination. A significantly prominent fibroblastic reaction, as well as dilated thick-walled vascular spaces in the dermis, occurred in one animal, giving the lesion a hemangioma-like appearance. One squamous cell carcinoma originating from the border of an ulcer of the skin was observed in one animal.

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TABLE

Number and types of tumors

Group	Treatment	Total No. of Animals	No. of TBA	% of TBA	Total No. of Grossly Verified Tumors	Number of Histologically Verified Tumors					
						Papillomas	Hemangiomas	Keratoacanthomas	Fibromas	Squamous Cell Carcinomas	Fibrosarcomas
A	DMBA once	40	—	—	—	—	—	—	—	—	—
B	UV light once	40	—	—	—	—	—	—	—	—	—
C	UV light 10×	40	2	5	2	2	—	—	—	—	—
D	Croton oil*	40	—	—	—	—	—	—	—	—	—
E	DMBA once followed by croton oil*	30	9	30	10	6	—	2	—	—	—
F	UV light once followed by DMBA once and croton oil*	30	12	40	26	8	1	2	—	1	1
G	UV light 10× followed by DMBA once and croton oil*	30	9	30	12	7	—	—	2	1	2
H	DMBA once followed by UV light once and croton oil*	30	8	27	9	6	—	—	—	1	—
I	DMBA once followed by UV light 10× and croton oil*	30	3	10	4	—	—	—	—	—	—

TBA = tumor bearing animals.

* 0.02 cc twice a week for 30 weeks.

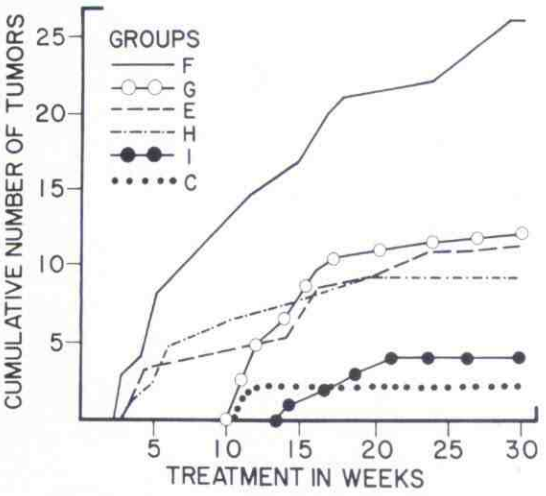

FIG. Number of grossly observed tumors.

When UV treatment was given 10 times prior to initiation followed by croton oil (Group G), ulceration and scarring of skin was present to some degree in all animals for the duration of the experiment. Stromal fibrosis was prominent; in 2 animals fibroma type lesions were observed. One fibrosarcoma also occurred.

UV light exposure once after initiation (Group H) produced a number of papillomas (Table, Fig.) morphologically similar to those previously described. When the initiation was followed by UV light 10 times (Group I), a small number of tumors was seen (Fig.) and these completely regressed.

DISCUSSION

The effect of pretreatment of the skin before the application of a carcinogen as orginally suggested by Mottram [1, 2] was also studied by Shinozuka and Ritchie [9], who found that pretreatment with croton oil caused a small increase in the number of papillomas. This was partly verified by Frei and Harsono [10]. Hennings, Bowden, and Boutwell [11] reported that pretreatment with croton oil caused a substantial increase in the number of papillomas per mouse as well as an acceleration in the rate of carcinoma formation. Pound and coworkers [12–15] showed that a single injury to mouse skin by scarification or chemical means shortly before initiation with urethane augmented the yield of skin tumors. They explained this as a probable result of an increased number of tumor foci associated with increased cellular proliferation and an increase in the number of cells replicating DNA at the time of initiation.

UV irradiation before application of a carcinogen [16] enhanced tumor production and to a greater extent premalignant "plaque formation." This was explained as depending on inhibition of hair growth by the UV light leading to a longer period of activity in situ of the carcinogen. Andreasen and Englebreth-Holm [17] and Borum [18, 19] have shown that tumors and premalignant lesions occur

more readily when the hair follicles are at the resting stage.

Another possible explanation for the stimulation of tumorigenesis in these circumstances would be initiation of tumor formation by UV light, giving rise to a summation effect [20]. This explanation, however, is not supported by experiments carried out in our laboratory which showed a lack of carcinogenic effect of UV light at a similar dose as that used in these experiments. These animals were observed for life. Rusch, Kline, and Baumann [21] found no increase in the number of tumors produced by repeated applications of 20-methylcholanthrene or 9,10-dimethyl-1,2-benzanthracene when the carcinogen treatment was preceded or followed by UV irradiation.

Previous studies indicate that short-term UV exposures applied within a few days after treatment with a carcinogen will not enhance skin tumor formation [16]. These studies in fact suggest that tumorigenesis may be inhibited by UV treatment. Other authors have also reported that exposure of skin to sunlight and fluorescent light reduced the number of chemically induced tumors [22, 23]. This phenomenon might be due to the oxidation of DMBA to a noncarcinogenic derivative caused by the light exposure. Photo-oxidation of carcinogenic hydrocarbons results in quinone-like products which are not readily bound to protein [24]. In support of this view, Miller has found that less hydrocarbon is bound to protein if mice are irradiated after the carcinogen is applied [25].

Another possible explanation is that UV light prevents tumor formation by destroying the latent tumor cells produced by the carcinogen. Large doses of a carcinogen applied on the skin are less effective in tumorigenesis than small doses [26, 27]. This has been explained as depending partially on the destructive effect of large doses of chemical carcinogens. Doniach and Mottram [22] suggested that their finding that sunlight inhibited chemically-induced skin carcinogenesis might be due to strong sunlight increasing the dermatitis caused by the photodynamic properties of the carcinogens.

The morphologic pattern of the neoplastic response varies greatly depending on the type of treatment. Tumors of mouse skin are epidermal in origin when the topical carcinogen [28] or ultraviolet light [20] is applied. In contrast, a 90 percent sarcoma incidence was obtained when the ears of mice were treated with UV light [29]. The occurrence of malignant tumors in mice given UV light 10 times and DMBA points to a summative effect, although UV light given 10 times is mildly tumorigenic by itself.

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