Radiation Induction of Cancer of the Skin

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ABSTRACT

The induction of epidermal tumors was studied using exposures to 25 kV

X-rays with or without subsequent exposures to 12-0-tetradeconyl phorbol-13

acetate (TPA) or ultraviolet radiation (UVR) 280-400 nm. Fractionation

regimens and total exposure upto 4000R produced no squamous cell carcinomas.

When these regimens were followed by TPA an incidence of about 80% was

obtained, and an incidence of 60% when UVR exposures followed the

X-irradiation. A dose-dependent increase in fibrosarcomas was found when

X-irradiation was followed by 24 weeks of topical treatment with TPA. These

results support the contention that UVR can enhance the expression of cells

initiated by X-rays. The experimental evidence is compared with the data

from the tinea capitis patients treated with X-rays. In C3HF/He male mice

exposed to 50, 100, 150 and 200 rads ^{137}Cs gamma rays the induction rate

for fibrosarcomas was 2.9×10^{-4} per cGy/per mouse. This result compares

with 2.5×10^{-6} transformations per surviving cell per cGy with 10T1/2

cells that are fibroblasts derived from C3H mice.

INTRODUCTION

The skin is exposed to various agents especially ultraviolet radiation

(UVR) yet the importance of interactions or co-carcinogenesis is only

becoming appreciated and future risk estimates must take interactions into account. For example, psoriasis patients treated with X-rays are at greater risk if they are subsequently treated with PUVA (Stern et al., 1979).

Despite a considerable body of work on the induction of skin tumors by ionizing radiation, particularly by Burns and Albert and their colleagues, (see Albert, 1976 and Burns and Albert, 1985 for references) and Hulse and colleagues (Hulse, 1962, 1967, 1969, 1980, 1983 and Papworth and Hulse, 1983), there has been little work on interactions of X-rays with other agents.

This paper describes preliminary results from experiments designed to investigate interactions and whether the relative refractoriness of the skin to the induction of cancer by ionizing radiation is related to the initial events or their expression. In addition, data obtained for the induction of fibrosarcomas in mice exposed to gamma rays is presented.

METHODS

Only a brief, but hopefully sufficient description of the methods is given here. The complete description, to be published elsewhere, will include the assays necessary to determine whether the fractionation regimens alter transmission, especially of UVR through the skin. The data for fibrosarcomas is derived from a larger experiment that will be reported elsewhere.

Experiment I: Interactions

Ionizing radiation

Mice were exposed on a turntable with a wide mesh wire cover to 250R twice a week; to give a total of 2, 4, 8 or 16 exposures at 77R/minute. The source of the 25 kV X-rays was a Norelco unit with a beryllium window tube.

The HVL was 0.08 mm Al. Dose rates were measured with a MDH X-ray monitor using a thin walled ion chamber.

After the end of the irradiation regimens mice received either no further treatment or one of the following: 1) 5 µg 12-0-tetradeconyl phorbol-13-acetate (TPA) either 2 or 3 times per week for 24 weeks. 2) Ultraviolet radiation.

Ultraviolet radiation

Mice were exposed to UVR from a Westinghouse FS40 sunlamp, that emits wavelengths between 280-400 nm at a fluence rate of 2.0 watts/sq m, 3 times a week for 24 weeks. The dose per fraction, 250 J/m^2, was less than 20% of the minimal erythema dose.

Experiment II: Fibrosarcomas

Gamma Radiation

Exposures to ^{137}Cs source at 31 rad/min were made with mice caged in individual plastic tubes rotated in the beam. Radiation measurements were made with high-energy Victoreen ionization chambers calibrated at the National Bureau of Standards.

Experiment I. Interactions

Animals and Tissue Examination

Female 10-week-old SKH:hr-1 mice were maintained 8-10 mice per cage and housed in a room with gold lights on 12 hr dark - 12 hr light cycle.

Mice were examined regularly and the date of appearance, site and number of skin lesions were recorded. The progress or regression was noted at two-week intervals. The data analysis was based on the number of mice bearing one or more tumors of a specific type. The type of epithelial tumor was determined histologically but for analysis the data for papillomas and

premalignant tumors were pooled and classified as benign. All the malignant

epidermal tumors were squamous cell carcinomas. Malignancy was determined by

the invasiveness of the tumors and metastases. All enlarged regional nodes

were taken for histology. The lungs were examined.

Experiment II: Fibrosarcoma Induction

Male C3HF/He mice were housed 5 per cage housed in a barrier facility.

Moribund animals were killed and all tumors and a spectrum of tissues were

taken for histological examination. Dermal fibromas and fibrosarcomas were

considered a single class of tumors.

RESULTS AND DISCUSSION

Induction of Skin Tumors by Soft X-rays

The incidences of the different tumor types induced by X-rays and

X-irradiation and TPA are shown in Table I. No epidermal carcinomas or

.sarcomas occurred in the groups of mice that received only X-irradiation. In

the X-irradiated groups a total exposure of 4000R was required to induce a

mere 6% of benign tumors. However, when the irradiation regimens were

followed with protracted treatment with TPA not only the incidence of benign

epithelial tumors was increased but also squamous cell carcinomas to 80%.

Metastases in regional lymph nodes were found in 12% of the tumor- bearing

mice. It is of interest that the TPA treatment influenced sarcomagenesis.

In the case of the X-ray plus UVR a marked enhancement of the X-ray-initiated

tumorigenesis was also found (Fig. 1).

The dose-response curve for the induction of squamous cell carcinoma by

X-rays plus TPA appear sigmoid with a rapid rise in incidence when dose

levels that induce tumors is reached. This characteristic of the

dose-response curves has been noted previously in mice (Papworth and Hulse,

1983) and rats (Albert et al., 1967; Burns et al., 1975).

Albert (1976) concluded that in general the data for induction of skin cancers suggested a dose-squared relationship.

Papworth and Hulse (1983) examined the model that might describe the data for the induction of skin cancer by radiation. Without assuming a threshold there was difficulty in fitting any of the models to their data. They suggested that the dose-response data were consistent with a non-threshold induction process if some factor(s) restrained the appearance of tumors; in other words, a non-threshold curve shifted to the right.

Consider the following; ionizing radiation, UVR and PUVA interact with DNA to induce the changes that can lead to a malignant phenotype of affected cells. However, cancer does not appear, or does so only after a considerable time. Both the length of the latent period and the magnitude of the doses required for skin cancer induction suggest two possibilities. First, further changes in the target cells are required for the full development and expression of the initial changes. Such changes might be caused by exposure to other exogenous agents, or by instability of the genome induced by the non specific damage from the initial exposures. Second, multiple factors such as cell-cell interactions and immune responses restrain or suppress any progression of the carcinogenic process. Subsequent exposures to one of a variety of agents, or the changes with age, affect the restraining factors and the cancer becomes overt.

When so-called promoters are used the treatment has to be protracted to be effective. That is also the case with UVR and PUVA. This fact must tell us something about the mechanism. If the mechanism involves the induction of further changes in the target cell the events must have a very low

probability. On the other hand if the mechanism involves interference with factors that are restraining the expression of the initial events then, intuitively, the requirement for protracted treatment would be expected.

Our finding that treatment with TPA, and UVR after X-irradiation markedly increased the incidence of carcinomas is consistent with the idea that the determining factor of the dose-response relationships in skin cancer is not the initial events but those that influence expression of the initial events. It has been shown previously (Fry et al., 1982) that the shape of the dose-response curves for induction of skin cancer by PUVA could be changed markedly by treatment with TPA, which was assumed to affect expression but not initiation.

All the experimental evidence suggests that skin has a remarkable capacity for restraining or suppressing the process of tumorigenesis without necessarily repairing the initiation events or removing the initiated cells.

The current findings with UVR are consistent with the interpretation that the incidence of skin cancer in the tinea capitis patients is influenced by the amount of exposure to UVR (Shore et al., 1984). Although the study is not complete the average follow-up is now 26 years.

Two pieces of evidence from the study of the tinea capitis patients treated with 120 kV X-rays indicate that sunlight enhanced the expression of X-ray initiated cells. First, forty of the former patients have had one or more basal cell carcinomas. Whereas, none of the 500 black patients have yet presented with a skin cancer. These findings, while based on a small population sample, are consistent with the suggestion that cancers did not appear in X-irradiated skin of the black patients because the melanin acted as a screen against the sunlight (Walther et al., 1981). Secondly, the yield

of cancers in the areas of skin in the white patients exposed to sunlight is over eight times greater in skin protected by hair.

The experimental results reported here appear to support the possibility that a synergistic interaction between ionizing and ultraviolet radiation is important mechanistically and in the estimate of risk of radiation-induced skin cancer (Bechtel et al., 1980.

In the C3H mice exposed to gamma radiation the tumor data indicate a shallow dose response over the 0-200 rad dose range. Assuming a linear dose response the tumor rate is 2.9×10^{-4}/rad which can be compared with the _in vitro_ transformation frequency per surviving cells of 2.5×10^{-6} reported by Hill et al., 1984. The results obtained by Hill et al. were with 10T1/2 cells derived from a C3H mouse embryo (Reznikoff et al., 1973). The sarcoma induction rate _in vivo_ would be congruent with the transformation rate _in vitro_ if only 10^2 cells were at risk in the mice. Intuitively we believe the number of cells at risk must be manyfold greater than 100. The suspected marked difference between the _in vivo_ and _in vitro_ results are not surprising since the 10T1/2 cells are a cell line. Perhaps of equal or greater importance is the fact that cell environment and cell-cell interactions are quite different for cells in culture from those in the organized dermal connective tissue. The explanation of the differences in the rates of malignant transformation presents a challenge and not a trivial one because an understanding of the differences could make the _in vitro_ data even more valuable.

Table I

Skin Tumors

After Exposure to X-rays and TPA

Treatment		Incidence %		
X-ray (R) (25 kV)	TPA 5 g	Benign Epithelial	Squamous Cell Carcinoma	Fibrosarcoma
-	2/wk 24 wks	0.0	0.0	0.0
2 x 250	2/wk 24 wks	0.0	0.0	0.0
4 x 250	2/wk 24 wks	2.2 ± 2.0	2.4 ± 2.0	0.0
8 x 250	2/wk 24 wks	67.0 ± 13	36.0 ± 9.0	13.0 ± 7.0
16 x 250	2/wk 24 wks	79.0 ± 70	82.0 ± 7.0	18.0 ± 6.0
16 x 250	----	6.0 ± 40	0.0	0.0

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FIGURE LEGEND

Fig. 1. Incidence of squamous cell carcinoma after exposure to 4, 8 and 16 fractions of 250R, 25 kV X-rays: o——o and to 2, 4, 8 and 16 fractions of X-rays followed by 72 exposures (3/wk, 24 wks) of 250 J/m^2 UVR (280-400 nm) o——o.

SKH: hr−1 ♀ mice
250 R 2/week
25 kV X rays

X ray + UVR
250 J/m²
3/week 24 weeks

250 J/m² 3/week alone
X ray alone

EXPOSURE (R)

SQUAMOUS CELL CARCINOMA
CUMULATIVE INCIDENCE %