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The Effect of Fractionation on Tumor Induction
and Hair Follicle Damage in Rat Skin¹

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Abstract

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Recovery with respect to tumor and atrophic follicle induction was measured in terms of the difference ($D_2 - D_1$) between the fractionated dose (D_2) and the single dose (D_1) which produced equal effects. An initial dose of 750 rads was given to the dorsal skin of male, albino rats when the hair follicles were in the resting phase. Thirty-one days later, again in the resting phase, groups of previously irradiated rats and comparable unirradiated rats were given the following doses: 0 rads, 750 rads, 1500 rads, 2250 rads, and 3000 rads. The rats were observed every 4 weeks for 80 weeks. At 80 weeks all skin lesions were examined histologically, and the incidence of atrophic hair follicles was determined by observation of epithelial mounts. The values of $D_2 - D_1$ for skin tumor induction and hair follicle atrophy were 650 rads and 700 rads respectively.

Key Words: fractionation, tumor induction, skin, rat

INTRODUCTION

Recovery is a general radiobiological phenomenon which has been studied extensively with respect to cell survival in vitro and to some extent in vivo, but relatively little is known concerning its role in carcinogenesis. For irradiation of rat skin with beta particles, Henshaw et al., reported that with daily fractions a total of 24,600 rep was required to produce the same tumor incidence as a single dose of 4600 rep (1). Similar results were obtained with x-rays by Zackheim (2) while Hulse, et al. showed fractionation had relatively little effect on tumor induction in mouse skin at doses that produced ulceration (3).

Atrophic hair follicles have been implicated in radiation carcinogenesis of rat skin because of the observation that the tumor incidence was roughly proportional to the incidence of atrophic follicles over a wide range of dose, energy, and LET values (4, 5, 6, 7). The proportionality either reflects a coincidental similarity between the dose-effect relationships or the atrophy is somehow involved in the tumor induction process.

Hair follicle atrophy is presumably the result of cell

lethality with inadequate regeneration. In the dose range up to the maximally carcinogenic dose, the skin injury is less severe than might be expected from the magnitude of cell killing estimated from in vitro survival curves which suggests that recovery, possibly including regeneration, has occurred (8).

Regenerative proliferation and intracellular recovery have been studied separately in the overall recovery of mouse skin (9). Intracellular recovery is generally a rapid process which is completed within 12-24 hrs (10), while proliferative repair is considerably slower, because it is dependent on the rate of cell division. As a first step in evaluating the recovery kinetics of skin tumor induction and hair follicle atrophy, recovery will be measured with sufficient time between fractions to permit repopulation to occur. In a subsequent experiment repopulation will be excluded by shortening the interval between fractions.

MATERIALS AND METHODS

As shown in Table I, 113 rats were irradiated initially with a surface dose of 750 rads at 28 days of age. At 59 days of age the previously irradiated rats and 76 unirradiated rats were irradiated with graded doses of 0 rads, 750 rads, 1500 rads, 2250 rads, and 3000 rads. The rats were CD strain obtained from

Charles River Breeding Farms, Wilmington, Mass.

The rats were exposed to electrons from a Van de Graaff accelerator. The energy of the electron beam was adjusted to give a depth-dose curve which was approximately linear with a maximum penetration of 1.0 mm. All dose values given are surface dose. The first fraction was applied to an area of 12 cm² (2 cm x 6 cm) of the dorsal skin. The area was marked with tattoos, and by the time of the second fraction it had grown to an average of 24 cm². The hair phase at the time of irradiation was determined by clipping the hair two days prior to irradiation and observing the regrowth.

The rats were observed weekly for the first 4 weeks after each irradiation, and subsequently the hair was clipped and observations were made every 4 weeks for 80 weeks. Skin lesions were noted and identified at each observation. At 80 weeks the experiment was terminated, and samples of each lesion were taken for histological examination. The appearance time of a given lesion was taken as the time it was first noted. The tumor incidence rate in each 4 week interval was the number of new tumors divided by the average number of rats alive during the interval. The tumor incidence was the cumulative sum of the

rates for each period.

At 80 weeks 3 skin samples with a total area of about 1 cm² were taken from within the irradiated area. Whole mounts of the epithelium were prepared by soaking these samples in 0.5% crude trypsin at 4°C for 18 hrs and removing the dermis. The resulting sheets of epidermis with hair follicles attached were stained with hematoxylin and Sudan III and mounted in glycerin jelly.

RESULTS

Table II shows the severity of the acute injury for each dose group. To obtain these numbers an arbitrary injury grading system was established as follows: 50% to 100% of the skin area involved in a wet ulcer-grade 4; 25% to 50%-grade 3; trace to 25%-grade 2; other (included loss of brown scales, sparse hair and out of phase hair growth)-grade 1 and none-grade 0. It is inappropriate to extrapolate between data points because of the arbitrary nature of the grading system. Nevertheless, for a given total dose the fractionated exposure pattern produced a consistently lower injury score. Only at 1500 rads single exposure and 2250 rads fractionated exposure were the injury scores approximately equal (1.0 and 0.9 respectively) which suggests a fractionated dose (D_2) minus single dose (D_1) value of about

750 rads.

The tumor incidence as a function of time after irradiation for single exposures at 59 days of age is shown in Figure 1. For 3000 rads and 2250 rads tumors first appear at 12-16 weeks, and thereafter the curves rise steadily to values of 4.5 and 6.2 tumors per rat respectively at 80 weeks. For 750 rads there was only one tumor which occurred at 48 weeks, while for 1500 rads there were two tumors which occurred at 56 and 72 weeks.

The tumor incidence as a function of time after the second fraction for the fractionated exposures is shown in Figure 2. For total doses of 3750 and 3000 rads the curves are essentially identical; tumors first appear at 12 weeks and thereafter the curves rise steadily to values of 3.6 and 4.1 tumors per rat respectively at 80 weeks. At 2250 rads the first tumor occurred at 28 weeks and the incidence at 80 weeks was 0.75 tumors per rat. For 1500 rads one tumor occurred at 72 weeks, while for 750 rads one tumor occurred at 56 weeks and another occurred at 60 weeks.

The tumor incidence at 72 weeks as a function of dose is shown in Figure 3. The curves in Figure 3 are fairly independent of time, but 72 weeks was chosen instead of 80 weeks to avoid a possible bias associated with the difficulty of finding and

and sectioning small lesions which occurred in the last 8 weeks of the experiment. The horizontal displacement of the curves is a measure of D_2-D_1 and has a value which ranges between 650 rads at an incidence of 1.0 tumor per rat to 900 rads at an incidence of 4.0 tumors per rat. These variations in D_2-D_1 are the result of differences in the final incidence levels at high (ulcerating) doses where considerable variation normally occurs; thus, the value at low incidence is the best estimate of the true D_2-D_1 value.

Figure 4 shows the atrophic follicle incidence (as a percent of the total number of follicles) plotted against dose for single and fractionated exposures. The horizontal displacement of the curves gives a value of D_2-D_1 which ranges from 700 rads at an incidence 5% to about 1000 rads at an incidence of 14%.

DISCUSSION

In order for the difference between the total fractionated dose (D_2) and the single dose (D_1) to be a measure of recovery, it is necessary that the susceptibility be constant (11). One obvious factor which may affect susceptibility is the hair cycle with its marked changes in proliferation rates. However, there was no evidence that the initial fraction affected the hair cycle.

By 50 days of age both the irradiated rats and the controls showed a full regrowth of hair. At 59 days of age, the time of the second fraction, over 90% of the rats showed no hair growth in both control and irradiated groups (Table III).

The approximate equality of the D_2-D_1 values for induction of tumors and atrophic follicles is similar to observations made previously, i.e., that a close dose-response similarity exists (4). The significance of the similarity is unknown, but its persistent occurrence in spite of changes in a variety of radiobiological parameters supports the conclusion that hair follicle atrophy is a factor in skin tumor induction.

The magnitude of D_2-D_1 in the present experiment is typical of values obtained when the endpoint is structural integrity. For mouse skin and intestine with ulceration as the endpoint, D_2-D_1 values were in the range of 450-500 rads (11, 12). Follicle atrophy is, of course, a structural defect and a high D_2-D_1 is perhaps not surprising. However, a similar high value for tumor induction could only have been predicted on the basis of the assumption that permanent structural defects enhance or cause tumor induction.

Regenerative repopulation could have contributed to D_2-D_1 ,

since the 31 days between fractions was more than sufficient for cell division to occur. Withers has shown with an in vivo cloning technique for mouse epidermal cells that D_2-D_1 increased at a rate of about 100 rads per day as a result of cell division (9). The occurrence of normal hair growth during the interval between fractions is a conclusive indication that vigorous cell division did occur in the follicles. The relative contribution of repopulation to the value of D_2-D_1 can be determined by reducing the interval between fractions and that experiment is currently in progress.

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FIGURE LEGENDS

Figure 1. The tumor incidence as a function of the postirradiation elapsed time for single doses.

Figure 2. The tumor incidence as a function of the postirradiation elapsed time for fractionated exposures. The first fraction was 750 rads and the total dose is indicated.

Figure 3. The tumor incidence at 72 weeks as a function of the total dose for single and fractionated exposures.

Figure 4. The incidence of damaged hair follicles at 80 weeks as a function of the total dose for single and fractionated exposures.

Table I. Protocol.

Dose Fractions (Rads)	Total Dose (Rads)	Number of Rats
750 + 0	750	20
750 + 750	1500	23
750 + 1500	2250	24
750 + 2250	3000	23
750 + 3000	3750	23
0 + 0	0	39
0 + 750	750	19
0 + 1500	1500	19
0 + 2250	2250	20
0 + 3000	3000	18

Table II. The acute skin injury.

Exposure Pattern	Injury Score
0	0.0
750 rads	0.0
1500 rads	1.0
2250 rads	1.8
3000 rads	2.9
750 + 0 = 750 rads	0.0
750 + 750 = 1500 rads	0.3
750 + 1500 = 2250 rads	0.9
750 + 2250 = 3000 rads	2.1
750 + 3000 = 3750 rads	3.6

Table III. Hair growth phase at time of irradiation.

Dose Pattern	Rats without hair growth between 26 and 28 days (%)	Rats without hair growth between 59 and 62 days (%)
Control	100	94
750 rads + 0 rads	100	95
750 rads + 750 rads	100	91
0 rads + 750 rads	100	94

Single Exposure

Figure 1

Fractionated Exposure

Figure 2

Figure 3

Figure 4