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Tumor Incidence in Rat Skin
Following Proton Irradiation in a Sieve Pattern¹

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Abstract

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The dorsal skin of 183 male, albino rats was irradiated with 8.0 MeV protons at various doses in a uniform pattern and at two doses in sieve patterns with pore diameters of 5.0 mm, 2.0 mm and 0.5 mm. The depth-dose curve of the proton beam was modified so that it was approximately linear with a maximum penetration of about 0.8 mm. Skin lesions were noted every 4 weeks for 80 weeks and at the end of the experiment were examined histologically. The results indicated that the sieve pattern did not significantly affect the tumor incidence per unit irradiated area or the incidence of damaged hair follicles at any pore size. This contrasts to the results of earlier experiments with electrons where a sieve pattern produced a significant protective effect at a pore diameter of 2.5 mm. Possible explanations for this discrepancy are discussed.

INTRODUCTION

In calculating the tumor risk associated with exposure to ionizing radiation, it is often necessary to take into account the effect of nonuniform dose distributions, since the dose may be localized as, for example, in the vicinity of small radioactive particles. There is evidence that tissue injury as measured by erythema or ulceration is reduced if either the size of the irradiated area is reduced or the spacing between irradiated areas is increased (1, 2).

In an initial experiment which involved the irradiation of rat skin with electrons, the tumor incidence per unit area was reduced when the radiation dose was given in a sieve pattern (3). It was not possible to ascribe this reduction unequivocally to a biological reaction within the tissue because of electron scattering which caused a dose gradient at the geometrical boundary of the pores. The gradient made it necessary to calculate within isodose zones the expected tumor yield based on the dose response curve for uniform irradiation.

Because of the uncertainties associated with this calculation and the progressive decrease of the protective effect with

increasing dose, the present experiment was undertaken in order to eliminate the effect of scattering by utilizing nonscattering radiation. Protons were selected because of the availability of an adequate beam and because the depth-dose curve could be modified to duplicate that of the electron beam used previously.

MATERIALS AND METHODS

Male, albino, CD strain rats from the Charles River Laboratory were irradiated at 58 days of age. To insure that the hair follicles were in the resting phase the hair was clipped three days prior to irradiation and rats showing hair growth on the day of irradiation were discarded. The rats were anesthetized with 15 mg/kg of chlorpromazine followed 1 hour later by 25 mg/kg pentobarbital and placed individually in small boxes. Through the lid of each box, a 3 x 8 centimeter area of the dorsal skin was exposed. The skin was held in place by double sided cellophane tape which covered the opening. The boxes were attached 12 at a time to the periphery of a circular table which rotated in a proton beam at 80 rpm for between 2 and 15 minutes. The table circumference was 293 centimeters.

The proton beam was produced by a tandem Van de Graaff

accelerator at the University of Pennsylvania, Philadelphia, Pennsylvania. The primary beam was far too intense to be used directly and was scattered by means of a 1.0 mil gold foil. The scattered beam emerged through an exit port centered at 23° from the beam axis (Figure 1). The exit port measured 5 centimeters horizontally and 2.5 centimeters vertically and was covered with a 2.0 mil mylar film. The port was located at a distance of 30 centimeters from the intersection of the primary beam with the gold foil.

The scattered flux was measured with a thin anthracene scintillation crystal which was centered on a backscatter port located at 160° from the beam axis. The flux at the exposure port was calculated from the flux at the backscatter port by means of the Rutherford scattering formula (4).

A spinning disc constructed with absorbers of various thickness was introduced into the beam between the exit port and the skin. The disc converted the natural 'Bragg curve' of the proton beam into a linear shape. In effect 21 individual Bragg curves of varying lengths were superimposed in such a way that the sum approximated a linear curve. Care was taken to insure

that the rate of rotation of the disc was not a rational multiple of the rate of rotation of the table. The depth-dose curve was measured with an argon-filled, parallel plate ionization chamber and is shown in Figure 2.

Once the flux at the center of the exposure port was known the average flux was calculated from the beam profile which was also derived from the Rutherford formula (Figure 3). The flux at the skin surface was determined by multiplying the average flux at the exposure port by factors representing the fraction of time spent in the beam per table revolution and the fraction of the beam passing through the spinning disc.

The flux at the skin surface was converted to dose rate by multiplying by the stopping power. Since the spinning disc superimposed all parts of the Bragg curve at the skin surface, the average stopping power was used. The range of the scattered beam in aluminum was found to be 115 mg/cm² which corresponds to a beam with an energy of 8.0 MeV (5) for which the published value of the average stopping power is 1.58×10^6 rad cm² (6).

The sieves were made of 0.11 mm thick brass and the pattern was such that each pore was at the center of a regular hexagon

with a pore at each vertex. As indicated in Table I, the three pore diameters were 5 mm, 2 mm and 0.5 mm for which the percentage open areas were 35%, 42.5% and 22.5%, respectively.

After the exposures the rats were observed and skin lesions were noted every 4 weeks for 80 weeks and at death or the end of the experiment each lesion was examined histologically. The cumulative tumor incidence per rat was obtained by adding in successive 4 week intervals the ratio of the number of new tumors to the average number of rats alive during the interval.

At the time of death whole mounts of the epidermis and hair follicles were made by incubating pieces of full thickness skin in 0.5% trypsin at 4°C overnight and then peeling the epithelium in a sheet from the dermis. The sheet of epithelium was fixed in formaline, stained with hematoxylin and sudan III, and mounted in glycerine jelly.

RESULTS

The tumor incidence per 24 cm² of irradiated skin versus postirradiation elapsed time for the various irradiation patterns is shown in Figure 4 for a dose of 1200 rads. Twenty-four cm² was the area of the irradiated skin for the uniform pattern at

the time of irradiation. Skin growth in the unirradiated rats increased this area to an average of about 35 cm² by the end of the experiment (7). However, since the skin growth could not be reliably measured for the sieve pattern, all results have been expressed in terms of the size of the original area. The curves for the sieve patterns are not significantly different than the comparable curve for the uniform pattern. At this dose the sieve pattern did not produce a reduction in the tumor yield per unit area at any of the pore sizes used.

At 1550 rads the results are similar to those obtained at 1200 rads, i.e., the sieve pattern had no effect on the tumor incidence per unit area. The incidence per unit area at 1550 rads versus postirradiation elapsed time for the 2.0 mm sieve pattern and the uniform pattern is shown in Figure 5. The data for the 0.5 mm and 5.0 mm sieve patterns were not included because the number of tumor bearing rats was too small for valid comparisons to be made. For a pore diameter of 2.0 mm the curve of tumor incidence per unit area versus time is nearly identical to the comparable curve for the uniform pattern.

The data are summarized in Table II which gives the number

of rats, the total number of tumors at 80 weeks, and the tumor incidence per 24 cm² with standard deviations which were calculated by assuming proportionality with the square root of the total number of tumors. In none of the sieve pattern groups was the tumor incidence per 24 cm² of irradiated area significantly different at the 5% confidence level than the corresponding incidence for the uniform pattern.

Figure 6 shows the tumor incidence for proton irradiation in a uniform pattern at 80 weeks versus the dose at a depth of 0.3 mm in the skin. In earlier experiments it was found that the tumor incidence depended strongly on penetration and that the penetration dependence disappeared when the incidence was plotted against the dose at 0.3 mm (8). By utilizing the latter finding, it is possible to make comparisons of the relative effectiveness of radiations having diverse penetrations. Thus, in Figure 6 results are included from earlier experiments with electrons and alpha particles for which the maximum penetrations were about 1.0 mm (9).

The slopes of the three curves are approximately equal but they are shifted relative to one another along the dose axis. The

magnitude of the shift gives the relative biological effectiveness (RBE) and its value is 2.7 for alpha particles with respect to electrons and in the present experiment is in the range 2.2-2.6 for protons with respect to electrons. The slopes are such that an increase in the dose by 50% more than doubles the tumor yield.

The number of damaged follicles per unit area with standard errors for the sieve irradiation patterns and the ratio of damaged follicles to tumors are shown in Table III. The number of damaged follicles per unit irradiated area was essentially constant except possibly at 5.0 mm pore diameter at 1200 rads where the damage was somewhat low. There was no tendency for the damage to decrease with decreasing pore size. The ratio of damaged follicles to tumors was between 1000 and 4000 which is the same general range as has been observed in earlier experiments with electrons and alpha particles (7, 9).

DISCUSSION

The conclusion that the sieve pattern failed to produce a reduction in the tumor incidence is based on a comparison of experimental groups in which the number of tumors did not exceed 22 and was somewhat less in some groups. In order to calculate

the statistical significance of the differences, it was assumed that the tumors were distributed as a Poisson distribution, and the total number of tumors was used as an estimate of the variance of the tumor distribution (10). Mortality was neglected as negligible, since there was 81% survival at 80 weeks when the experiment was terminated.

The experimentally observed difference (Table II) between the 2.0 mm sieve pattern incidence and the uniform pattern incidence at 1550 rads would be expected on a chance basis with a probability of 0.48. A reduction would have been significant at the 5% confidence level if 6 or fewer tumors had occurred in the sieve group. The occurrence of 6 tumors would have corresponded to a tumor incidence of approximately 0.44 tumors per 24 cm² which is 35% of the uniform pattern incidence. Similar calculations for the 1200 rad dose groups show that a reduction in the tumor incidence of the sieve pattern to about 21% or less of the uniform pattern incidence would have been significant at the 5% confidence level.

While the differences were not significant, the tumor incidence per 24 cm² at pore diameters of 5.0 mm and 2.0 mm was

actually greater than for the corresponding uniform pattern. The lack of even a suggestion of a reduction makes it unlikely that the sieve pattern produced any degree of protection at all. Certainly a reduction in the tumor incidence by a factor of 8 or more as occurred in the earlier electron experiment at 1500 rads and a pore diameter of 2.5 mm did not occur in the present experiment. An explanation is needed for the apparent discrepancy that a sieve pattern protected against skin tumor induction for electrons but not for protons.

One can argue that the calculation of expected tumor yield per pore in the original electron experiment, based as it was on an incompletely known dose-response curve for uniform irradiation, might have been inaccurate. However, some of the uncertainties of the calculation are eliminated if the scatter zone is neglected and the effective pore diameter is assumed to be 1.6 mm within which the dose variation was less than 5% (3). Even within this smaller region the sieve pattern tumor incidence per unit area was $1/4$, instead of $1/8$, as great as the uniform pattern incidence for a surface dose of 1500 rads.

Two factors among the physical characteristics of the

radiations might be the basis of an explanation of the discrepancy: (1) differences in scattering and (2) differences in linear energy transfer (LET). Electrons have a relatively low LET but scatter considerably. Protons do not scatter but have an LET perhaps 100 times as great as that of the electrons. Fractionation experiments show that lethal intracellular damage produced by high LET radiation is less susceptible to repair than similar damage produced by low LET radiation (11). If the damage produced by protons were irreparable, sieve pattern protection would not be expected. An LET effect can be distinguished from a scattering effect by utilizing low energy x-rays which have a relatively low LET but do not scatter appreciably.

A preliminary experiment was performed in order to evaluate the sieve effect for 50 KVP x-rays on hair follicle damage. Six to 8 rats were irradiated with a surface dose of 2500 R in each of three sieve patterns at pore diameters of 2.5 mm, 5.0 mm and 10.0 mm. The incidence of damaged hair follicles was determined on whole skin mounts at various times between 14 and 36 weeks postirradiation and the results are summarized in Table IV.

The data show that the incidence of damaged hair follicles

per unit area did not decrease as the diameter of the pore was reduced from 10.0 mm to 2.5 mm. The 10.0 mm pore is large enough to be considered equivalent to a uniform irradiation pattern and the lack of any decrease in damaged hair follicles per unit area means sieve protection did not occur.

A tumor experiment similar to the one just described but extending to smaller pore sizes is in progress, and until tumor incidence data are available it cannot be definitively concluded that the tumor incidence per unit area would similarly show no reduction with the pore size. However, in several previous experiments under similar conditions, hair follicle damage has been a good indicator of the tumor incidence (7).

If a difference in scattering is the correct explanation, the mechanism is unknown. However, the phenomenon is sufficiently interesting that it would seem worthwhile to repeat and extend the earlier electron experiment by inducing scattering artificially with a nonscattering type of radiation.

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FIGURE LEGENDS

- Figure 1 - A schematic diagram of the scattering chamber and the irradiation apparatus.
- Figure 2 - The depth-dose curve for the scattered proton beam showing the approximately linear shape caused by the spinning absorber disc.
- Figure 3 - The beam profile calculated with the Rutherford formula. Values are relative to the central point and the average value is 1.18 as shown.
- Figure 4 - The cumulative tumor incidence per 24 cm² of irradiated skin with respect to elapsed time after irradiation for the various irradiation patterns at a surface dose of 1200 rads.
- Figure 5 - The cumulative tumor incidence per 24 cm² of irradiated skin with respect to the elapsed time after irradiation for the various irradiation patterns at a surface dose of 1550 rads.

Figure 6 - The cumulative tumor incidence at 80 weeks after irradiation in a uniform pattern versus dose at 0.3 mm for protons, alpha particles and electrons (9).

Table I Sieve Characteristics

Pore diameter (mm)	0.5	2.0	5.0
Distance between hole centers along rows (mm)	1.0	3.0	8.0
Distance between rows (mm)	0.9	2.4	7.0
Fractional open area	.225	.424	.350

Table II Comparison of tumor responses for uniform and sieve irradiation patterns.

Surface Dose (rads)	Pattern	Tumor Incidence at 80 Weeks Tumors/24 cm ²	Total Number of Tumors	Number of Rats
1550	uniform	1.25 $\pm$.37	11	9
	5.0 mm sieve		3	12
	2.0 mm sieve	1.60 $\pm$.34	22	35
	0.5 mm sieve		1	12
1200	uniform	1.04 $\pm$.39	7	8
	5.0 mm sieve	1.70 $\pm$.44	15	28
	2.0 mm sieve	1.20 $\pm$.36	11	23
	0.5 mm sieve	0.73 $\pm$.36	4	28
2400	uniform	2.80 $\pm$.80	15	6
1000	uniform		0	6
650	uniform		0	5
450	uniform		0	6
200	uniform		0	6

Table III A comparison of the tumor incidence and hair follicle damage in the sieve pattern groups.

Dose (rads)	Pattern	Damaged Follicles per 24 cm ²	Tumor Incidence at 80 Weeks	Ratio of Damaged Follicles/Tumor
1550	5.0 mm sieve	2400 $\pm$ 560	1.60	1750
	2.0 mm sieve	2800 $\pm$ 600		
	0.5 mm sieve	2900 $\pm$ 520		
1200	5.0 mm sieve	1600 $\pm$ 280	1.70	950
	2.0 mm sieve	3100 $\pm$ 800	1.20	2600
	0.5 mm sieve	2700 $\pm$ 680	0.73	3700

Table IV The relationship of damaged follicles per pore and pore size for 50 KVP x-rays (surface dose - 2500 r)

Pore Diameter (mm)	Number of Rats	Pore Area (mm ²)	Damaged Follicles per Pore	Damaged Follicles per Unit Area
10.0	6	78.5	110 $\pm$ 30	1.4 $\pm$ 0.4
5.0	6	19.6	30.8 $\pm$ 7.0	1.6 $\pm$ 0.4
2.0	3	4.9	7.8 $\pm$ 2.2	1.6 $\pm$ 0.5

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6