RBE FOR LATE SOMATIC EFFECTS IN MICE IRRADIATED WITH 60 MeV PROTONS RELATIVE TO X-RAYS[1)]

E. B. Darden, Jr., N. K. Clapp, R. S. Bender,[2)] M. C. Jernigan, A. C. Upton[3)]

Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830, U.S.A.

1. INTRODUCTION

The objective of this study, a joint project of the U. S. Atomic Energy Commission and the National Space Agency, was to examine the relative biological effectiveness (RBE) of energetic protons for the induction of somatic effects in a mammal (the mouse) following whole body irradiation. The proton energy used approximates the mean energy for proton spectra accompanying solar events. The mouse was chosen to permit uniform irradiation of the body with medium energy protons and the use of statistically significant numbers of animals. Extensive background information on late effects in the particular strain chosen (RF) was already available from recent studies in this laboratory with several other types of ionizing radiation [1-3)]. This presentation summarizes effects on longevity and the incidence of the major (mostly) neoplastic diseases. Detailed reports are being published sequentially as various portions of the study are completed. They include to date the methodology of the proton irradiation and the induction of lens opacities[4-5)]. One of the intentions was to include a thorough pathological study although budget cuts have delayed completion of the histological evaluation and conclusions.

2. MATERIALS AND METHODS

About 3000 8-week-old RF female mice were set up in replicates for treatment as shown in Table 1. The proton doses were 7% lower than corresponding X-ray values because of subsequent dosimetric corrections.

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1) Research jointly sponsored by the National Space Agency, and the U.S. Atomic Energy Commission under contract with the Union Carbide Corp.

2) Present address: Physics Department, The Citadel, Charleston, South Carolina, U.S.A.

3) Present address: State University of New York, Stony Brook, New York, U.S.A.

The mice, treated in replicates, received single whole-body irradiation with 60-MeV protons or 300 kVp X-rays or were sham-irradiated. Proton irradiation was given at the Oak Ridge Isochronous Cyclotron in a specially designed beam facility providing uniform exposure to monoenergetic protons over a 10 X 12 cm field[4]. Up to 4 mice, restrained in a horizontal position in individual rotating plastic tubes, were irradiated simultaneously with the beam incident to the body axis as shown in Fig. 1. The rationale of using 8-week-old mice for whole body irradiation is illustrated in Fig. 2. Protons of this energy have a range of 30 mm in tissue, and the internal diameter of the restraining tube was 24 mm. Accordingly, the proton path length across the body was on a relatively flat portion of the Bragg curve. The dose rate was maintained at approximately 100 rads/min to agree with the X-ray dose rate.

Table 1

Number of mice at risk[a]

Absorbed dose[b] (rads)	Protons	X Rays
0	526	
50	315	315
100	310	308
200	266	262
300	266	255
400	160	159

[a] Mice surviving $\geq$ 30 days post treatment.

[b] Corresponding proton dose 7% less.

Mice were exposed to X-rays from above in similar restraining tubes arranged radially in a plastic scattering dish on a revolving turntable. The exposure factors were: 300 kVp, HVL about 1 mm cu, distance to center of mouse, 60-90 cm. The exposure rate was 60-90 R/min and uniform along the body axis within 6%. A conversion factor of 0.95 rads/R was used to convert exposure to absorbed dose.

Following irradiation, the mice (in groups of 8) were returned to their cages and allowed to live out their life span except for periodic examinations of the lens. Thorough necropsies were performed on mice (30-day survivors) picked up moribund or dead. Tissues were taken for histological examination not only to confirm observations made at gross necropsy but also to detect more subtle changes observable only through histopathological study. Gross and histopathological information, recorded on specially designed necropsy cards, has been stored in a time sharing computer. Analysis is now in process with the assistance of Dr. John M. Yuhas of the Biology Division.

3. RESULTS

The major late somatic effects are life shortening, induction of neoplasia, particularly leukemia, and glomerulosclerosis. The results, based on all of the data for any one endpoint, in general, have supported our preliminary findings.

Life shortening increases with dose of either radiation up to about the 300-rad level with no further increase at 400 rads (Fig. 3). X-rays appear to be slightly more effective than protons although some of the 95%-confidence intervals overlap. If one assumes strictly for purposes of comparison that the dose response is linear for both radiations over the rising portion of the curve, then life shortening amounts to about 60 days per 100 rads of protons and 75 days per 100 rads of X-rays. The corresponding RBE for the protons is therefore 0.8.

Late somatic effects caused by ionizing radiation usually refer to induced changes in latency, incidence, or severity of characteristic diseases rather than the induction of entirely new lesions. Studies of the comparative effectiveness of radiation for induction of late effects is difficult because the observed differences are often smaller than corresponding differences between irradiated and unirradiated groups. We attempted to make the base for comparison of pathology as broad as we could, obtaining histological data in addition to gross data on 75-85% of the mice in each dose group. The results presented in Table 2 are based on histologically examined animals only.

The principal neoplasms in this strain are certain leukemias, tumors of the lung and, in female mice, the ovary. A variety of tumors were identified in other organs, especially in old animals, irrespective of irradiation, but in frequencies that were too low to be separately analyzed. Two of the leukemias, thymic lymphoma and myeloid (granulocytic) leukemia, are radiation-induced. Both are relatively acute, fatal diseases, and account for the bulk of the early mortality ($\leq$ 600 days of age) from sublethal irradiation. The other leukemias, mostly reticulum cell sarcoma and lymphatic leukemia, develop relatively slowly and are frequently seen in older animals, regardless of treatment.

Thymic leukemia appears and reaches its maximum incidence a little earlier in the life span than myeloid leukemia, but each disease has essentially run its course by late middle age (550-600 days) in all irradiated groups. The incidence at each dose level is therefore a reasonable measure of the ability of the radiation to induce either disease. The incidence of thymic leukemia increased with dose up to 300 rads as shown in Table 2. X-rays appear to be a little more effective than protons especially at higher dose levels, but the difference is of doubtful significance considering the 7% more dose received by the X-ray groups. In the case of myeloid leukemia, the incidence also increased with dose up to 200 rads with X-rays more leukemogenic than protons (RBE less than 1), but the difference vanished at the higher dose levels.

The combined incidence figures shown for the two diseases are an expression of radiation-induced leukemogenesis as a whole and show a positive correlation with dose through the 300-rad level, with protons slightly but consistently less effective than X-rays. At doses $\geq$ 200 rads nearly half of all deaths occur from radiation-induced leukemia. The RBE of the protons for induction of thymic lymphoma and myeloid leukemia, alone and combined, is essentially unity over the dose range observed. The combined incidence of the other leukemias (primarily reticulum cell sarcoma) was highest in the nonirradiated animals and displayed a consistent negative dose response (Table 2).

Lung tumors were common in all dose groups but were relatively small in size, and barring occasional indirect complications, were seldom implicated as a cause of death. The vast majority were nonmetastasizing

adenomas with a few adenocarcinomas. Radiation does not appear to be tumorigenic with regard to either type, singly or combined (Table 2). On the other hand, radiation is known to be strongly tumorigenic for the ovary as the data shows, even at the lowest dose level (50 rads). Protons seemed more effective in this respect than X-rays considering the 7% discrepancy in corresponding doses but the difference is marginal. The RBE of protons for induction of ovarian tumors over the dose range used is not significantly greater than 1.

The major nonneoplastic disease in these mice is glomerulosclerosis. It was present at least to a slight degree in almost every mouse older than 600 days. Classifying the lesion as "slight," "mild," or "severe" in the histologically examined mice revealed no radiogenic effect. The "severe" cases occurred in highest frequency about the same time (500-600 days) in all groups and, as shown in Table 2, declined at higher dose levels with no consistent difference between radiations.

These results, showing a similar effectiveness of 50-400 rad doses of 60-MeV protons and X-rays for life shortening, et al., support our previously reported finding of an RBE of 1 for induction of lens opacities[5].

4. CONCLUSIONS AND DISCUSSION

The small difference in life shortening effectiveness of 60-MeV protons and X-rays in the mouse appear to be largely the result of differences (also relatively small) in their leukemogenic effectiveness. Unlike the risk of radiation-induced leukemia which diminished sharply in old age, the risk of other diseases appeared by and large to be age related, i.e., they increased throughout life. Only in the induction of ovarian tumors did radiation add to the risk. The radiation-induced leukemias are also unique in that the cause of death is usually apparent. In other deaths, especially in old animals, the actual cause is often in doubt because of the presence of more than one potentially lethal disease. It seems likely that at times disease interaction rather than a single specific disease is implicated and some analysis, as yet incomplete, is being done on the occurrence of certain suspicious disease combinations.

It appears unlikely that some unexpected or dramatic delayed effect from medium energy proton irradiation will emerge from our data, results on the whole being quite similar to those obtained with X-rays. In one sense, the similarity is to be expected since the maximum (exit) value of LET for 60-MeV protons after traversal of the mouse body (assuming wet tissue) was less than 3 KeV/micron (Fig. 3) and therefore the average LET was equal to or less than that for X-rays. However, from a microdosimetric point of view, the distribution of absorbed energy from the two radiations in tissue is not the same and this, as well as local morphological differences, might conceivably affect the biological outcome. For example, for equivalent whole-body exposure of the mouse to X-rays and 60-MeV protons, the energy absorbed in the marrow cavities might be appreciably higher with X-rays because of photoelectron production in adjacent bony tissue. One may speculate on the relation to the suggested greater relative effectiveness observed with X-rays for the induction of myeloid leukemia.

In summary, we conclude that medium energy proton irradiation is no more effective, and on the whole, probably less effective than conventional X-radiation for the induction of late radiation effects in the mouse. The results of the lens study also support this conclusion[5]. Obviously, it is hazardous to extrapolate these results to protection of human beings against space radiation. One point might be worth emphasizing, however. In a proton event, the exposure, by being protracted over a period of many hours or even days, instead of being delivered within a short interval, might permit more recovery and repair, thus diminishing further the biological effectiveness of low LET protons for the induction of delayed effects.

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Table 2

Radiation level and incidences of major diseases in histologically examined mice

Disease	Dose Groups											
	0 Rads	50 Rads[a]		100 Rads		200 Rads		300 Rads		400 Rads	
		P	X	P	X	P	X	P	X	P	X
Thymic lymphoma	5[b]	9	9	11	14	26	24	34	39	31	43
Myeloid leukemia	1	2	7	6	12	16	23	16	16	13	11
Thymic lymphoma + myeloid leukemia	6	11	16	17	26	42	47	50	54	43	54
Other leukemias	46	44	42	39	41	31	25	25	20	20	21
Lung tumors (adenomas and carcinomas)	26	26	28	30	19	18	26	21	16	17	18
Ovarian tumors (all types)	3	40	42	54	51	48	38	41	36	39	36
Glomerulosclerosis (severe)	34	31	31	36	31	21	25	29	19	22	26
No. of mice histologically examined	427	269	262	258	259	212	219	221	227	127	122

[a]Proton dose about 7% less in all dose groups.

[b]Percentage showing the disease (rounded to nearest percent).

FIGURE LEGENDS

Fig. 1. Rotator loaded with mice in position for proton exposure. Beam enters obliquely from right. (from reference 4).

Fig. 2. Bragg ionization curve for 60-MeV protons. The solid curve is the calculated energy loss for a single particle in soft tissue. The open circles are experimental data. (from reference 4).

Fig. 3. Life shortening in mice irradiated with 60-MeV protons and X-rays. Life shortening is expressed as difference in mean survival time with respect to that of unirradiated controls (620 days). Vertical bars are 95% confidence limits.

Fig. 1

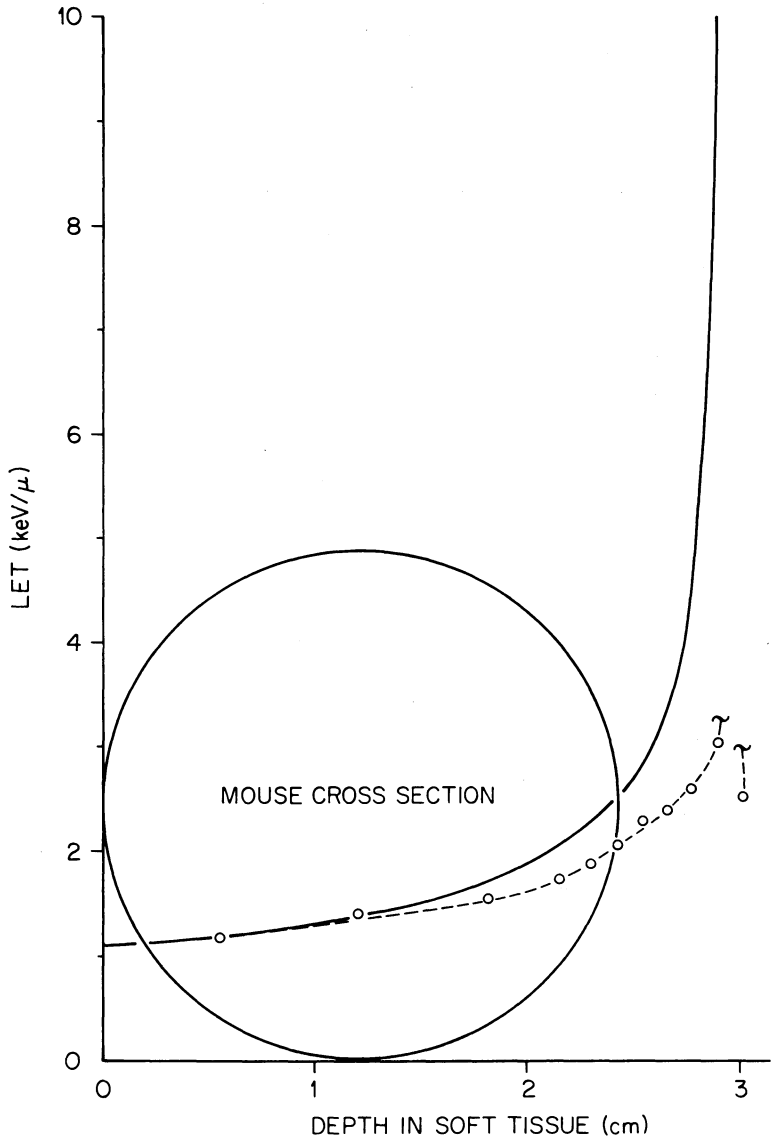
10
8
6
4
2
0
LET (keV/μ)
MOUSE CROSS SECTION
0
1
2
3
DEPTH IN SOFT TISSUE (cm)

Fig. 2

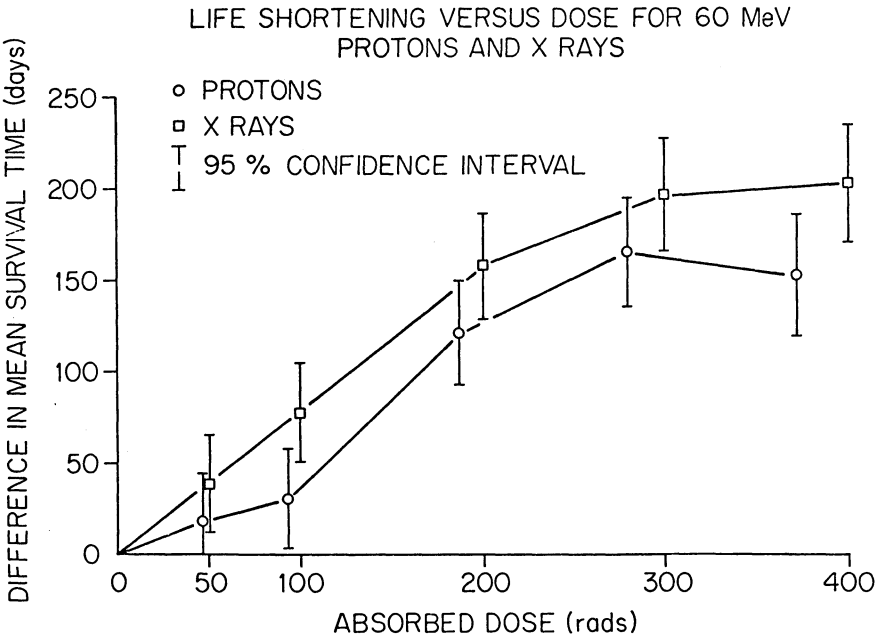
LIFE SHORTENING VERSUS DOSE FOR 60 MeV
PROTONS AND X RAYS
PROTONS
X RAYS
95 % CONFIDENCE INTERVAL
DIFFERENCE IN MEAN SURVIVAL TIME (days)
250
200
150
100
50
0
0
50
100
200
300
400
ABSORBED DOSE (rads)

Fig. 3

DISCUSSION

Paper : Life shortening and late pathological effects in mice irradiated with 60 MeV protons and with X-rays

BURCH: What was the average value of LET, throughout the body of the mouse, for the proton irradiations?

DARDEN: The average value of LET (averaged over volume) was 1.45 keV/micron.