Paradoxical Effect of Radiation on Tumor Incidence in the Rat: Implications for Radiation Therapy

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ABSTRACT

The high incidence of leukemia in the Fischer rat is reduced by radiation to an incidence below that seen spontaneously. Fractionating the radiation decreased this effect. In contrast, mammary tumors increased with dose until reaching a plateau at the highest doses. Fractionation had little effect. These results are consistent with a hypothesis suggesting that tumor incidence due to radiation is the result of competing processes of tumor induction and cell killing.

INTRODUCTION

Radiation is known to be a potent carcinogen. Some have suggested that, in the establishment of radiation limits for large populations, it is prudent to assume that tumor induction is linear with dose. This assumption has been criticized at very low dose levels, with some suggesting a greater and others a lesser likelihood for tumor incidence. What seems clear, in both the laboratory and the clinic, is that the curve is not linear at high doses. Studies of myeloid leukemia in the RF mouse have indicated that the frequency of this tumor reaches a maximum at about 300 rads and then there is a subsequent decline (17, 18). Similar findings are noted in the CBA mouse (7). In humans, there is no evidence of tumor induction when the pelvis is given high doses of radiation for carcinoma of the uterine cervix (2, 14) as compared to the increased incidence of tumors seen when similar treatment is given but to lower doses for benign pelvic tumors (14, 15). These findings have been explained by assuming that the curve of tumor induction is a composite of a linear increase in tumor induction on the one hand and exponential killing of the target cells on the other (6, 8) (Chart 1). If the tumor incidence is the result of these competing processes, then when the spontaneous incidence of the tumor is high and the cells are quite sensitive to radiation one would expect that the tumor incidence should rise with radiation dose very little, if at all, before decreasing. This decrease may extend to below the unirradiated level. Thus, high doses of radiation in this circumstance may have a protective effect. In contrast, if the tumor has a low spontaneous incidence and/or if the cell survival curve has a large shoulder or a shallow slope, the tumor induction should increase with dose for a greater extent before cell killing becomes dominant. Fractionation of the radiation might have different consequences in these 2 situations. In the former circumstance, fractionation should increase the likelihood of tumor because it allows for less effective radiation cell killing. In addition, cell proliferation may occur between fractions resulting in a greater number of the transformed cells and therefore a higher tumor incidence. On the other hand, if the tumor has a low spontaneous frequency or the tumor cells are less radiosensitive, then fractionation should reduce tumor incidence because the effective dose of radiation is lower. This is related to where on a biphasic tumor induction curve (Chart 1) the dose to be divided is located. If it is on the initial ascending portion of the curve, fractionation makes the effective dose lower, and thus the tumor incidence will decrease. If the dose is on the descending portion of the curve, reducing the effective dose by fractionation may increase the tumor incidence.

The Fischer rat affords an excellent opportunity to study these predictions in the same animal because it has a high incidence of a characteristic leukemia (9) and a very low incidence of mammary tumors. Studies of both single and fractionated radiation doses were used in animals followed until death, at which time all tumors were scored and life shortening was taken into account. The results obtained support a hypothesis suggesting that tumor incidence due to radiation is the result of competing processes of tumor induction and cell killing. The leukemia incidence was decreased by radiation to well below the spontaneous level; this decrease was less when the dose was fractionated. Mammary carcinoma, on the other hand, was increased with dose, and this was not significantly ameliorated by fractionation.

MATERIALS AND METHODS

Fifty inbred Fischer rats (Dunning's 344 Fischer line), 2 months old, were randomly allocated to each of 6 groups. Radiation was given using a 250-kV X-ray machine, half-value layer of 1.5 mm copper, with a dose rate of 115 to 120 rads/min. Different groups of animals received 0, 60, 120, 240, 120 x 2, and 60 x 4 rads. The fractionated radiation programs were: (a) 120 rads, 3 days, 120 rads; (b) 60 rads, 2 days, 60 rads, 2 days, 60 rads, 3 days, 60 rads. Following irradiation, the animals were caged at 2/cage and followed carefully for survival. At death, postmortem studies were performed, and the incidence of leukemia and breast neoplasms were scored.

The leukemia has been described elsewhere (9). The disease occurs late in the rat's life with about 25% of observed animals developing the disease. The leukemia is characterized by an enlarged spleen with infiltration of both spleen and liver. Characteristic mononuclear cells and nucleated RBC appeared in the peripheral blood where leukemia cell counts may reach 150,000/cu mm. The diameter of the leukemic cell measures 15 to 20 μm, but the cell of origin is uncertain, and thus this is best called a mononuclear cell. At death, there is frequent involvement of the mesenteric lymph nodes, while significant bone marrow involvement occurred in only 20% of the animals.

Breast tumors have been described by others (10, 12). In this series, there were 83% adenofibromas, 8% carcinomas, 5% sarcomas, and 4% fibromas. The numbers of malignant tumors appeared to follow the overall tumor incidence, but the incidence is too low for a separate
evaluation of malignant tumors; thus, all breast tumors are considered. Calculation of the actuarial likelihood for survival or leukemia induction were performed using the technique of Berkson and Gage (1) corrected for intercurrent death (1, 5). This allowed for the life shortening due to radiation. Breast tumors were scored grossly at death and verified histologically. The number of tumors rather than the number of animals with tumors are presented, since on occasion animals presented with more than one tumor (166 tumors in 154 animals). Presentation either way does not alter the qualitative nature of the data.

RESULTS

Chart 2 shows the actuarial survival curves for the control and the single-dose groups. There seems to be no discernible effect of 60 rads on life span. Perhaps there is a slight effect at 120 rads; however, there is a 3.5-month reduction in median life span in the 240-rad group. Fractionation of the 240-rad dose appears to have no effect on this life span reduction (Chart 3).

Leukemia in the control group first appears at 20 months and gradually increases with an actuarial likelihood reaching 51% by 32 months. A similar curve is 60 and 120 rads (Chart 4); however, there appears to be a clear reduction in leukemia incidence at 240 rads. Since this is an actuarial plot, the effect is independent of life span. The actual percentage of leukemia for each group is shown in Table 1. Chart 5 shows the final actuarial incidence of leukemia as well as the actual incidence of leukemia plotted as a function of dose and shows that with increasing dose of radiation there is either a plateau or slight rise in leukemia with 60 rads; after that, however, the incidence seems to fall, and at 240 rads it is well below the spontaneous incidence. Dividing the dose into smaller fractions appears to increase the incidence of leukemia. The actual incidence in increasing from 2% at 240 rads x 1 to 8% at 120 rads x 2 to 12% at 60 rads x 4 (Table 1). Further, with increasing fractionations, the tumor appears to occur somewhat earlier, as shown in Chart 6.

Breast tumors found grossly at death were confirmed histologically and are plotted against months after radiation in Chart 7. There appears to be an increase in number of breast tumors as well as an earlier time of presentation of such tumors as the dose is increased. These tumors are quite uncommon in the control animals and appear only late in life. The final total accumulated number is shown in Chart 5. The increase with dose appears to approach a plateau between 120 and 240 rads. Table 1 shows that this number seems to decrease.
somewhat when the dose is fractionated; therefore, fractionated radiation does not increase tumor induction. This is opposite to that seen with leukemia.

DISCUSSION

The expected life shortening is noted in the group receiving 240 rads. Dividing the dose into 2 or 4 fractions appears to make little difference to this life shortening. The actuarial method of determining incidence of leukemia allows correction for the life shortening seen. Table 2 and Chart 5 present the data for both actual and actuarial determinations. The acute leukemia has a very high spontaneous incidence. It appears only slightly increased with the 60-rad dose; at doses above this, there is a decrease so that at the 240-rad dose level the incidence is below that seen spontaneously. While life shortening is accounted for by the actuarial method, since no animals lived beyond 30 months it is possible that tumors might have appeared later had the animals survived. At any age, however, the incidence is lower than in the controls. The actuarial probability of leukemia at this dose is 8%, and the simple incidence is 2% as compared to 52 and 28%, respectively, in the control animals. Similar findings for reticulum cell sarcoma were observed by Ullrich and Storer (16). This tumor incidence was also very high in the controls and decreased with radiation dose even when this was adjusted for life shortening.

The breast tumors seen are similar to those described by Shellabarger et al. (3, 4, 10, 12, 13) in Sprague-Dawley rats. They have shown these to increase with radiation dose and to appear earlier in the life span of the animals. In our studies, the incidence seems to plateau between 120 and 240 rads. Perhaps at higher doses this curve will begin to decrease as was observed in the curves for leukemia. The tumor incidence curves for leukemia and breast neoplasia appear quite different. The leukemia with a high spontaneous incidence is actually decreasing at the highest doses of radiation, while the breast cancer with a low spontaneous incidence increases with radiation dose. Both can be fitted to the theoretical curve (Chart 1) of tumor induction but at different positions on the abscissa. Leukemia with an initial high incidence may be close to the maximum induction possible. Hematopoietic and lymphocytic cells and their malignant variants are quite sensitive to radiation. Thus, for such a tumor there can be little if any rise in tumor incidence with dose and the dominant effect of radiation is to reduce the tumor incidence when doses resulting in significant cell killing are administered. There is a low spontaneous incidence of breast neoplasia in this rat strain. Increasing doses of radiation increase this tumor frequency. At the largest doses administered, there appears to be a plateau suggesting a balance of tumor induction and cell killing. Perhaps had higher doses been administered, the descending limb of Chart 1 would have been seen.

Because of these differences, fractionation has contrasting effects on tumor incidence. Fractionation essentially lowers the effective dose and therefore can be considered as changing

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Actuarial</th>
<th>Simple</th>
<th>Total no. of breast tumors</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>52</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>60 rads</td>
<td>70</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>120 rads</td>
<td>39</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>240 rads</td>
<td>8</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>120 rads × 2</td>
<td>31</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>60 rads × 4</td>
<td>17</td>
<td>12</td>
<td>28</td>
</tr>
</tbody>
</table>

Chart 5. Simple and actuarial leukemia incidence and number of breast tumors as a function of radiation dose.

Chart 6. Cumulative probability of leukemia mortality for differing radiation doses. x2, x4, number of fractionated doses.

Chart 7. Number of breast tumors seen on groups of rats receiving differing doses of radiation. •—•, control; x—x, 60 rads; O—O, 120 rads; •—•, 240 rads.
the position of the curves in Chart 5, moving then toward a lower value. For leukemia, this raises the incidence as seen from the data where simple incidence rises from 2 to 12%. For breast tumors, this decrease in effective dose lowers slightly the number of breast tumors observed from 37 to 28. Shellabarger et al. (11) conclude that there is no decrease in the number of mammary tumors produced by 500 R when delivered in a fractionated form, although the data suggest such a trend.

Since the leukemia has a high spontaneous incidence reaching almost 52% at 32 months, it is difficult to raise this incidence. The predominant effect of radiation is to destroy cells which are progenitors of the leukemia and therefore decrease the incidence. Breast tumors, on the other hand, have a low spontaneous incidence which is increased by radiation dose, and the effects of radiation killing of progenitor cells of the cancer are not really seen until the plateau at 240 rads. These results indicate that while radiation is a powerful tumor-inducing agent its administration may not increase tumor incidence and in certain circumstances may decrease the likelihood of tumor, especially at higher doses. This may be important when one considers the decrease in tumor induction seen with the high doses used in the radiation therapy (2, 14) as compared to that seen with doses formerly used for benign disease (14, 15). Extrapolation of tumor incidence curves from low to high doses will be quite misleading and will tend to markedly overestimate tumor likelihood.

REFERENCES

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