The Dose-response Relationships for Tumor Induction after High-LET Radiation.

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This paper presents a review of several studies conducted in our laboratory to examine the carcinogenic effects in mice of high-LET radiation and, for comparison, of low-LET reference radiation. For some specific end-points the following conclusions can be formulated: i) the dose-response curves for myeloid leukemia and malignant lymphoma can be interpreted in terms of induction and inactivation; in particular, the data confirmed that a linear dependence of the induction on dose is adequate to describe the response to fission neutrons, while a pure quadratic dependence is consistent with the experimental data for low-LET radiation; ii) in the liver, a marked age-dependence was demonstrated for radiation-induced tumors with a much higher susceptibility in young than in old mice; also for these tumors the dose-effect curves can be described by a linear and a quadratic relationships for high and low-LET radiation, respectively; iii) data on ovarian tumor induction suggested threshold-like dose responses: these peculiar shapes as well as the absence of a clear radiation quality dependence of the curves are difficult findings to explain using a simple model of radiation action, and they might better be related to a non-stochastic effect of hormonal imbalance following irradiation.

INTRODUCTION

It is widely recognized that, in absence of human data, a substantial source of information on the shape of the dose-response relationships for cancer induction after irradiation remains the experimentation with animals irradiated whole-body.

In regard to the physical variables that have been studied for their capacity to influence the induction of tumors in animals, the dose, dose-rate, dose fractionation, and radiation quality have received special attention. It appears difficult, however, to generalize the effect of these factors on the various tumor systems: the peculiarity of each system and the complexity of other biological factors which act at the tissue level are such to prevent any unified description, and it appears, therefore, uneasy to interpret the animal data by simple models. However, in very general terms the data reported in the literature up to now are consistent with the assumption that the dose-response curves are linear or linear-quadratic for high- and low-LET radiation, respectively. In addition, high-LET radiation is more effective in inducing tumors, and RBE values increase at progressively lower doses (UNSCEAR 1986)\textsuperscript{1}.

Furthermore, age at the time of irradiation and sex are among the biological variables
that have a major influence on the induction of tumors in experimental animals (Fry, et al. 1989). However, information on these topics are up to now quite insufficient.

These considerations have inspired a great interest in gaining more knowledge on the effects of radiation doses, particularly after high-LET radiation, with the aim to contribute to risk estimates. The present study is a comprehensive evaluation on the results obtained in our laboratory over the last ten years from several experiments on the late somatic effects after both neutrons and X rays in BC3F1 and CBA/Cne mice.

**MATERIALS AND METHODS**

The animals used and methods applied in these studies will not be described in any detail as they have been extensively reported elsewhere (Covelli, et al. 1989). Neutron irradiated BC3F1 mice were whole-body exposed at the fast neutron reactor RSV TAPIRO at Casaccia. Female BC3F1 mice received single acute doses of 1.5 MeV neutrons produced by a 3 MeV van de Graff accelerator at the Joint Research Center (JRC) - Euratom (Ispra, Italy). BC3F1 and CBA/Cne male mice receiving single whole-body X-ray irradiation were exposed to a deep therapy unit operated at 250 kVp, HVL=1.5 mm Cu.

Details of the neutron and X-ray dosimetry, as well as irradiation protocols and procedures, animal follow-up and pathology, and the methods of data analysis, were presented previously.

**RESULTS**

*Malignant lymphoma*

The tumor appears with a very high spontaneous incidence in non-irradiated animals (~60%) and the relationship between incidence and X-ray irradiation seems to indicate the presence of a plateau extending from 0 to a dose about 4 Gy followed by a fairly steep decrease with the dose. As far as neutrons are concerned, only a progressive negative slope of the dose-response curve for this tumor is evident.

Using current dose-response relationships for the tumor incidence data, good fits were obtained if including cell inactivation. In particular, the X-ray data were best fitted with a quadratic model

\[ I(D) = (a_0 + a_2D^2)e^{-\lambda D} \]  

including a factor for exponential cell inactivation, acting on both the spontaneous and the irradiation induced component with a probability \( \lambda = 0.70 \pm 0.04 \text{ Gy}^{-1} \).

For fission neutrons, the data were best fitted by a linear activation model modified by exponential cell inactivation. The value of the inactivation probability \( \lambda \), obtained from the fit, was \( 1.0 \pm 0.5 \text{ Gy}^{-1} \).
Myeloid leukemia

In a previous paper (Di Majo, et al. 1986)\textsuperscript{4}, we have reported that CBA/Cne male mice irradiated with single acute doses of X rays display myeloid leukemia (ML) at all radiation doses tested with a maximum at 3 Gy followed by a reduction in frequency at higher doses. The highly curvilinear relationship between dose and incidence is in good agreement with Mole’s experimental results (Mole, et al. 1983)\textsuperscript{5}). In particular, the experimental points were best fitted by a function of the type:

\[ I(D) = a_2D^2e^{-\lambda D} \]  \hspace{1cm} [2]

with an induction term quadratic with the dose multiplied by an exponential inactivation factor.

In a more recent review of several experiments carried out with BC3F\textsubscript{1} male mice exposed to single acute X-ray and fission neutron doses (Covelli, et al. 1989)\textsuperscript{3}), we have noted that in over 600 of these mice no spontaneous incidence of ML was present, as in the case of CBA. The X-ray data were well described by the same expression valid for CBA mice, i.e. by a quadratic induction term corrected by an exponential inactivation factor. The induction observed after fission neutrons was consistent with the expected slope corresponding to a linear induction coupled with exponential inactivation (Fig. 1), with an equation of the type:

\[ I(D) = a_1De^{-\lambda D} \]  \hspace{1cm} [3]

Fig. 1. Age-adjusted percent incidence of myeloid leukemia after whole-body exposure to 250 kVp X rays (\textbullet) and fission neutrons (\textdagger). Fitted curves correspond to equation 2 and 3, respectively (see text). Bars are standard errors.
Liver tumors

The influence of fission neutron irradiation on the development of liver tumors in BC3F1 male mice irradiated at 3 and 19 months of age or in utero at 17 days post coitum (p.c.) is shown in Fig. 2.

In the dose range where the risk appears to increase as a function of the dose increase, the data points after neutrons were well fitted by a function of the type:

\[ I(D) = a_0 + a_1D \]  

were the values of the linear parameter \( a_1 \) per 100 mice span from a maximum of 118 per Gy for prenatal irradiation \( (P=0.43) \) to a minimum of 3 per Gy \( (P=0.93) \) for mice irradiated at 19 months of age, with an intermediate value of 35 per Gy \( (P=0.98) \) for young-adult mice. A similar inference can also be drawn from the X-ray dose-response data. Here the dose dependence is purely quadratic and the values of the \( a_2 \) parameters are fairly close for the in utero and 3-month-old irradiated animals (1.7 and 1.2 per Gy\(^2\), respectively), while \( a_2 \) is not significantly different from zero for old mice.

Ovarian tumors

Previous studies in this laboratory on BC3F1 female mice (Covelli, et al.)\(^6\) suggested that the ovary is very sensitive to tumor induction after X-ray irradiation with a maximum incidence of over 50% at a dose of 75 cGy. The data here reported (see Table), which include points at very low X-ray doses, indicate that no significantly increased incidence is
Table 1. Incidence of Ovarian Tumors in Whole-Body Irradiated Mice

<table>
<thead>
<tr>
<th>Type of radiation</th>
<th>Dose (cGy)</th>
<th>No. of mice</th>
<th>Tubular adenoma</th>
<th>Granulosa cell tumor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Luteoma</th>
<th>Mixed tumor</th>
<th>Papillary cystadenoma</th>
<th>Teratoma</th>
<th>Total&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Percent Incidence&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
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<td>X-rays</td>
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<td>335</td>
<td>27</td>
<td>4(1)</td>
<td>3</td>
<td></td>
<td></td>
<td>34(1)</td>
<td>10 (10)</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>97</td>
<td>5</td>
<td>4(2)</td>
<td></td>
<td></td>
<td></td>
<td>9(2)</td>
<td>9 (8)</td>
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<tr>
<td></td>
<td>8</td>
<td>79</td>
<td>13</td>
<td>2(1)</td>
<td>1</td>
<td>1</td>
<td></td>
<td>17(1)</td>
<td>20 (15)&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>16</td>
<td>52</td>
<td>21</td>
<td>6(3)</td>
<td>3</td>
<td>4</td>
<td></td>
<td>34(3)</td>
<td>60 (45)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>32</td>
<td>56</td>
<td>22</td>
<td>25(11)</td>
<td>2</td>
<td>3</td>
<td></td>
<td>52(11)</td>
<td>80 (47)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>128</td>
<td>59</td>
<td>7</td>
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<td>5</td>
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<td>51</td>
<td>6</td>
<td>15(3)</td>
<td>5</td>
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<td></td>
<td>29(3)</td>
<td>51 (61)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Neutrons</td>
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<td>4</td>
<td>7</td>
<td>2</td>
<td>48(1)</td>
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<td>90</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>19(1)</td>
<td>21 (26)&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>16</td>
<td>48</td>
<td>15</td>
<td>1</td>
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<td></td>
<td>16</td>
<td>33 (42)&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Numbers in parentheses refer to malignant tumors.
<sup>b</sup> Numbers in parentheses are age-adjusted incidences.
<sup>c</sup> P<0.001.
<sup>d</sup> 0.10>P>0.05.
<sup>e</sup> P=0.16.
observed at doses below 8 cGy with a steep rise at doses slightly above. Consequently, the current models of radiation action do not appear adequate to describe the data at low doses. For instance, the best fit in the 0–16 cGy dose region obtained with the linear no-threshold model corresponds to a probability as low as 0.012. Therefore, the existence of a threshold dose at about 8 cGy cannot be excluded.

As far as 1.5 MeV neutrons are concerned, a significant increase of incidence with respect to the control animals is first observed in mice receiving a dose of 8 cGy. Over the dose range examined, i.e. 0–16 cGy, a variety of dose-response models adequately describe the data. In fact, the application of a quadratic dose-response model provides the best fit to the experimental points (P=0.98), although linear or linear-quadratic expressions fit the data equally well (P=0.92 and P=0.96, respectively). However, as previously observed for X rays, a threshold-model cannot be excluded to describe the dose-response relationship for neutrons. In fact, a linear relationship fits well the data (P=0.58) with a threshold-dose of 6 cGy (Covelli, et al. 1988).

CONCLUSIONS

The studies reported before have been conducted with the objective to improve our knowledge on the type of the dose-response relationships for radiation carcinogenesis in mice, and possibly determine the RBE of radiation of different qualities. The tumor induction data reported here have indicated that the bone marrow, the liver, and the ovary of the mice tested are among the tissues with a certain degree of susceptibility to radiation carcinogenesis. Within the limits of resolution of the present analysis, these results have also confirmed that a linear relationship appears to be adequate for a conservative description of the dose-effect curves relative to radiation carcinogenesis after exposure to low doses of neutrons, while a pure quadratic dependence is consistent with the experimental data obtained for the above tissues with low-LET radiation. In particular, this point is confirmed by the analysis of tumors of the hemolymphopoietic tissues, i.e. lymphomas and myeloid leukemia. For these tumors the dose incidence curves can be interpreted as the result of the competition of transformation and inactivation of the bone-marrow hemopoietic stem cells, which are believed to be the target cells for transformation. This might also suggest that at the cellular level the mechanisms responsible for these two types of tumors are not different.

Another information which stems from the present analysis, and appears of great interest, is that the susceptibility to radiation induction of selected tumors by fission neutrons decreases in the old age. In fact, liver tumor induction in BC3F1 mice is clearly age-dependent with higher susceptibility in young than in old mice. To our knowledge, this is one of the rare demonstrations of a significant influence of the age at exposure in experimental radiation carcinogenesis. Furthermore, the dose-effect curves for this tumor can be described again by a quadratic and a linear relationship for low and high-LET radiation, respectively. An attempt of including an exponential inactivation factor $e^{-D/D_0}$ in the dose-effect relationship was also done, using $D_0$ values coherent with previous
observations for X rays and for neutrons, to obtain a model for the entire phenomenon. However, the results were unsatisfactory and, therefore, the reduction of hepatocellular tumor frequencies observed at the highest doses tested could not be interpreted on the basis of a pure radiobiological model of radiation action. It might be related to transformable target saturation, and/or associated, at least in animals irradiated as young-adult, with a higher risk of death with a severe degenerative disease of the kidney (nephrosclerosis). The incidence of this disease increased consistently and significantly at doses above 4 Gy of X rays and 1.79 Gy of neutrons, with a shortening of the latent period in respect to that of liver neoplasms.

The experimental data on ovarian tumor induction suggest a threshold like dose-response. This shape, as well as the absence of a clear radiation quality dependence of the curve, are difficult findings to explain using a simple model of radiation action and they might better be related to a non-stochastic effect of hormonal imbalance following irradiation. The hypothesis that a certain degree of oocyte killing is essential to begin a sequence of events leading to the development of ovarian tumors also appears fascinating. Recent epidemiological analysis conducted on the atomic bomb survivors in Hiroshima and Nagasaki (Tokuaka, et al. 1987) are consistent with the hypothesis that also in humans, radiation injury to the ovary with a secondary excess of gonadotropic hormones is an important causative factor in the development of ovarian neoplasm.

Finally, the estimation of neutron RBE for the different types of tumor analyzed in the present study is a difficult job, particularly at low doses. The different nature of the neutrons and X-ray dose-response relationships suggests that these values cannot be derived as the ratio of the induction coefficients. However in the case of myeloid leukemia an RBE value derived at the lowest neutron dose of 0.17 Gy is around 4, which is consistent with the previous values reported in the literature on the same subject.

In the liver, a marked age-dependence was demonstrated for radiation induced tumors with a much higher susceptibility in young than in old mice: also for these tumors the dose-effect curves can be described by a quadratic and a linear relationship for low and high-LET radiation, respectively; age-dependence also appears to exist for neutron RBE relative to X rays for the induction of liver neoplasms. In fact RBE values for prenatal irradiation, i.e. 28±9 at 0.09 Gy, is two times higher than for young adult animals irradiated at comparable low doses, i.e. 13±1 at 0.17 Gy.

Finally, if a threshold-dose exists for the induction of ovarian tumor by either low- or high-LET radiation, calculations of neutron RBE might appear meaningless. However, to obtain information with regard to the different effectiveness of the two radiation qualities in the dose-region where the effect is most pronounced, the slopes of the two linear fits (P=0.65 and P=0.58 for X rays and neutrons, respectively) have been compared and yielded a value close to unity (RBE=0.8±0.2) For the ratio. This is in agreement with the RBE value around 1 mentioned by Dobson and Straume (1982) for ovarian tumors in the mouse in a comparable dose region.

In conclusion, more experimental data appear necessary to refine the dose-response already obtained in the different tissues and organs at risk following whole-body irradiation.
and they become essential to support explanation of mechanisms and dose-effect relationships in man.

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REFERENCES