Tumourigenesis by Partial Body X-irradiation in Mice

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ABSTRACT. The aim of the present experiment was to investigate the late effects of partial body X-irradiation on mice. A total of 428 ddY/SLC female mice 10 weeks old were assigned to the following four groups: (1) head exposure with 950 rad, (2) trunk exposure with 950 rad, (3) lower body exposure with 950 rad, (4) unirradiated control. Mean after survival times with their standard errors were as follows: 430±13 days for head exposure, 354±8 days for trunk exposure, 435±13 days for lower body exposure, 472±14 days for control. Life shortening was 9 percent by head exposure and 25 percent by trunk exposure. Irradiation to the lower body did not induce statistically significant life shortening. Head exposure with 950 rad induced pituitary tumours. Trunk exposure with 950 rad induced ovarian tumours and reduced malignant lymphomas. The reduction may be attributable to the extensive life shortening after trunk exposure. An increase of lung tumours after trunk exposure was not statistically significant. Lower body exposure with 950 rad did not change the tumour spectrum of the control group—KEY WORDS: mouse, ovarian tumour, partial body irradiation, pituitary tumour, radiation tumourigenesis.


Adult animals which have received ionizing radiation doses, which are not acutely lethal, usually appear to recover from early radiation syndromes within a month or two after exposure. As the animals grow older, however, they may have a higher incidence of certain tumours than unexposed animals. Experimental evidence for tumourigenesis by ionizing radiation [2, 19, 21, 29, 31, 37, 39] has been used for the estimation of human risks from radiation together with human data. Experiments by partial body irradiation may simulate some sorts of accidental exposures in humans. On the other hand, it is interesting to know if any indirect effect on shielded tissues from irradiated tissues exists in tumourigenesis. In previous reports the authors have reported on tumourigenesis [1, 22] and life shortening [23] in mice by X-irradiation. Whole body irradiation with 570 rad induced malignant lymphomas and trunk irradiation with 760 rad induced ovarian tumours. Lower body exposure to 760 rad did not cause the tumour spectrum to differ from that of the unexposed. On the whole, partial body irradiations with 760 rad have shown somewhat rather smaller effects than expected [22]. The present experiment was designed to study life shortening and tumourigenesis by partial body irradiation with the higher dose of 950 rad.

MATERIALS AND METHODS

The mice used throughout the experiments were ddY/SLC virgin female mice which were outbred in the Shizuoka Laboratory Animal Cooperative Association, Japan and purchased at the age of 6 weeks. The mice were housed five to a cage and provided with food (MB-1, Funabashi Farm Co., Japan) and tap water \textit{ad libitum}. They were kept in “conventional” conditions.
Cage groups were not disturbed. The room temperature was kept at 24±1°C.

When they reached the age of 10 weeks, they were assigned to the following groups:

1. Head exposure.
2. Trunk exposure (forelegs, chest and abdomen).
3. Lower body exposure (pelvis, hind legs and tail).
4. Unirradiated control (only anaesthetized).

Since it was not feasible to obtain the requisite number of mice all in one shipment, the population was set up in a number of duplicate groups. Total number of mice used for each experimental group was as follows: 112 mice (3 duplicates of 56, 30 and 26 mice) in head exposure, 112 mice (3 duplicates of 30, 59 and 23 mice) in trunk exposure, 119 mice (3 duplicates 30, 30 and 59 mice) in lower body exposure, and 85 mice (2 duplicates of 29 and 56 mice) in unirradiated control. A roentgenogram of the mouse demonstrating the irradiated regions was presented in a previous paper on acute mortality [24].

Radiation exposure was at 10 weeks of age. During irradiation the mice were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (0.1 mg/g body weight). At each time 15 mice were irradiated. The X-ray machine was operated at 200 kVp, 20 mA, with a filter of 0.5 mm Cu and 0.5 mm Al. Dosimetry was done with Radcon Model 575. The dose-rates were 51 to 57 rad/min. Shieldings in the partial body irradiation were composed of lead 5 mm thick. The absorbed dose in the shielded area was a few per cent of that in the irradiated area.

All the mice were checked daily to obtain material for post mortem examination. The animals were followed until natural death. When they were found dead, their corpses were necropsied as soon as possible and fixed in an isotonic neutral formal. Tissues with macroscopic abnormalities were dissected for histological examination and processed in the usual manner. All the sections were stained with hematoxylin and eosin and some of them, when necessary, with reticulum or other special stains. In addition to various types of tumours, inflammatory diseases were examined on the sections.

Student's $t$-test or Welch's test was performed to analyze mean survival times. If the variances of the two groups to be compared were not significantly different, Student's $t$-test was used. If there was a significant difference between the variances in the two groups, Welch's test was used. The observed disease incidences were calculated as follows.

$$\text{Incidences} = \frac{\text{No. of mice with the disease}}{\text{No. of mice used}} \times 100$$

The disease incidences were analyzed with the $\chi^2$ test corrected by Yates' method in their contingency tables.

The experiments reported in the text were carried out in National Institute of Radiological Sciences and a part of statistical analyses was carried out in Faculty of Veterinary Medicine, Hokkaido University. The experiments started in 1976 and ended in 1985.

RESULTS

Life shortening: Survival curves are plotted against days after irradiation in Fig. 1. The life shortening caused by trunk exposure was extensive, while head exposure induced approximately the same survival curve as that caused by lower body exposure. When the data are plotted on normal probability graph paper, it results in nearly straight lines. This indicates that the individual life spans distribute approximately in normal distributions.

Data on mean survival times are summarized in Table 1. The mean survival time is
defined as mean life expectancy at 10 weeks of age when the mice were exposed to X-rays. The mean survival times of mice with tumour(s) and without tumours are shown in addition to the pooled mean survival times. The life shortening in the pooled data was 9 per cent by head exposure and 25 per cent by trunk exposure. The life shortening induced by the trunk exposure was extensive in both the mice with and without tumours. In contrast, irradiation of the lower body with 950 rad showed no life shortening with statistical significance.

Disease incidence: Tumours observed at autopsy were roughly classified as follows:
1. Pituitary tumours
2. Ovarian tumours: granulosa cell tumour, luteoma, adenocarcinoma, embryonal carcinoma and cystadenoma
3. Lung tumours
4. Malignant lymphomas: lymphosarcoma, reticulum cell sarcoma, lymphocytic leukemia and monocytic leukemia
5. Mammary tumours: adenocarcinoma and adenocanthoma
6. Other tumours: adrenal cortical tumour, hepatoma, fibrosarcoma,

Table 1. Mean survival times in days after partial body irradiation

<table>
<thead>
<tr>
<th>Area irradiated</th>
<th>Dose (rad)</th>
<th>Number of mice</th>
<th>Mean survival time±SE in days with tumour(s)</th>
<th>without tumour</th>
<th>pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>950</td>
<td>112</td>
<td>457±16(59)</td>
<td>399±20(53)*</td>
<td>430±13*</td>
</tr>
<tr>
<td>Trunk</td>
<td>950</td>
<td>112</td>
<td>371±10(75)**</td>
<td>320±14(37)**</td>
<td>354±8**</td>
</tr>
<tr>
<td>Lower body</td>
<td>950</td>
<td>119</td>
<td>450±16(69)</td>
<td>414±21(50)</td>
<td>435±13</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>85</td>
<td>476±16(57)</td>
<td>465±25(28)</td>
<td>472±14</td>
</tr>
</tbody>
</table>

Level of significances in comparison with control.
* 0.01 < p < 0.05.
** p < 0.01.
Numbers in parentheses are numbers of mice with or without tumour.
Table 2. Observed disease incidences (%) by partial body X-irradiation with their standard deviations

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Control</th>
<th>Irradiated at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Trunk</td>
</tr>
<tr>
<td>Pituitary tumour</td>
<td>0(0)</td>
<td>10.7±2.0(12)**</td>
</tr>
<tr>
<td>Ovarian tumour</td>
<td>3.5±2.0(3)</td>
<td>1.8±1.3( 2)</td>
</tr>
<tr>
<td>Lung tumour</td>
<td>29.4±4.9(25)</td>
<td>21.4±3.9(24)</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>28.2±4.9(24)</td>
<td>17.9±3.6(20)</td>
</tr>
<tr>
<td>Mammary tumour</td>
<td>5.9±2.6(5)</td>
<td>9.8±2.8(11)</td>
</tr>
<tr>
<td>Other tumour</td>
<td>8.2±3.0(7)</td>
<td>8.0±2.6( 9)</td>
</tr>
<tr>
<td>Inflammatory diseases</td>
<td>27.1±4.8(23)</td>
<td>42.0±4.7(47)*</td>
</tr>
<tr>
<td>Others and unknown</td>
<td>5.9±2.6(5)</td>
<td>5.4±2.1(6)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate numbers of mice with the disease.
Level of significances in comparison with control.
* 0.01<\(p\)<0.05.
** \(p\)<0.01.

Table 3. Numbers of mice died with reticulum cell sarcoma

<table>
<thead>
<tr>
<th>Area irradiated</th>
<th>Abdomen</th>
<th>Mediastinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Trunk</td>
<td>3**</td>
<td>1</td>
</tr>
</tbody>
</table>

Level of significance in comparison with control. \(**\) \(p\)<0.01.

The observed incidences of diseases are shown in Table 2. The inflammatory diseases are mostly lung abscesses in all the groups. Head exposure with 950 rad induced pituitary tumours and inflammatory diseases. The life shortening by head exposure primarily comes from mice without tumours (See Table 1) and the increase of the inflammatory diseases may contribute to the life shortening (See Table 2). Trunk exposure with 950 rad induced ovarian tumours and decreased the incidence of malignant lymphomas. The decrease of malignant lymphomas is primarily due to a decrease of reticulum cell sarcomas in the abdomen, as shown in Table 3. The observed reticulum cell sarcomas are characteristic of tissues described by Dunn [6] as type A reticulum cell neoplasm. The increase of lung tumours resulting from trunk exposure was not statistically significant with a 5% level of significance. Lower body exposure did not change the tumour spectrum of the control group. The sum of the percent incidences in each group exceeds 100% since some mice had two or more diseases.

The age distribution of deaths with lung tumours is shown in Fig. 2. The trunk exposure obviously accelerated the appearance times of lung tumours. The decrease of deaths with lung tumours at 600 days after trunk exposure may be due to the extensive life shortening in the group. The age distribution of deaths with malignant lymphomas is shown in Fig. 3. The decrease of the incidence beyond 450 days after trunk exposure may also be due to the extensive life shortening.

DISCUSSION

The mean survival time of the control in the previous report [22] was 484±13 days, which was fairly well reproduced as 472±14 days in the present report. The life shortening after head or trunk exposure in the
present report was larger than that in the previous report owing to the increase of the dose delivered. However, for unknown reasons, the life shortening after lower body exposure was not statistically significant in the present report. Data on atomic bomb survivors in Hiroshima and Nagasaki have shown that life shortenings are attributable to neoplastic diseases and that non-neoplastic diseases do not contribute to the life shortening [12]. On the other hand, some data on mice of the SAS/4 and RF strains showed that the oncogenic effects of whole body irradiation did not entirely account for its life shortening effect [15, 39]. Data in Table I in the text also show that there is life shortening in mice without tumours after trunk exposure. A reason for that may be an earlier appearance of the inflammatory diseases (mostly lung abscesses) in the group. Other data on BC3F1 male mice after whole body irradiation have shown that the life shortening below 400 rad is exclusively attributable to neoplastic diseases [5].

The observed incidence of pituitary tumours caused by head exposure was 10.7 per cent with 950 rad as shown in Table 2. The authors' previous report [22] had shown that head exposure with 760 rad induced pituitary tumours with 4.3 per cent of incidence in the same strain of ddY/SLC female mice and that no spontaneous pituitary tumours was observed in unirradiated control. Namely the pituitary tumours seem to increase with dose in the strain. It is generally difficult to induce pituitary tumours by whole body irradiation but Sasaki et al. observed the induction of pituitary tumours in B6WF1 [19, 21] and B6C3F1 [20] female mice by irradiating in the late fetal period or by irradiating neonatally. Diethylstilbestrol induced pituitary tumours in AXC female rats with an observed incidence of 84 per cent [25].

Ovarian tumour is one of the most easily radiation induced tumours in mice. A continuous gamma exposure during the entire life span induced ovarian tumours with an incidence of 100 per cent in LAF1 mice [16]. A single X-ray dose of 800 R delivered to
the head and lumbar areas of young LAFl mice also induced ovarian tumours with an incidence of 100 per cent [3]. Dose effect curves for ovarian tumours have a steep rise in lower dose regions and then a gradual increase or a plateau beyond about 100 rad [4, 35]. In the much higher dose region, for instance beyond 600 rad, the incidences begin to decrease [15, 19]. The 950 rad to the trunks of ddY/SLC mice induced ovarian tumours with an incidence of 13.4 per cent as shown in Table 2 and the dose might be higher than the optimal dose to obtain the highest incidence. The authors' previous report [22] had shown that trunk exposure with 760 rad induced ovarian tumours with 13.2 per cent of incidence in the same strain of ddY/SLC mice where their spontaneous incidence was 1.7 per cent. Komuro [13] had irradiated ddY/F mice with 130 R or 260 R of X-rays at whole body and observed 39.4 per cent of ovarian tumours during 18 months after the irradiation, while no ovarian tumour was observed in unirradiated control. The data on ddY mice suggest that the strain is sensitive to induction of ovarian tumours by ionizing radiation. X-irradiation at 16–18 days post coitum [21] or at birth [10, 19, 38] also induced ovarian tumours in mice.

Malignant lymphomas decreased following trunk exposure as shown in Table 2, reconfirming previous data [22]. The decrease was caused by a decrease of reticulum cell sarcoma in the abdomen. The decrease was also observed in BC3F1 [4] and RFM [33] mice after whole body exposure.

Lung tumours were slightly induced in young BALB/c female mice by whole body irradiation [36]. X-ray irradiation induced lung tumours in B6WF1 [19, 21] and B6C3F1 [20] mice when they were irradiated in the late fetal period or at birth. Generally, it is hard to induce lung tumours in young adult mice by whole body irradiation. The dose-effect curve of lung tumour induction in terms of observed incidence is almost flat [35] or declines with the dose [15, 26]. The decrease of lung tumours in the irradiated group to below the control level is sometimes explained by so-called "competing risks". Whole body exposure with several hundred rad induced many thymic lymphomas in most strains of mice and their latent periods were rather short. On the other hand, lung tumours are relatively late occurring tumours. Accordingly, if most mice die from the thymic lymphomas, there is little chance left to observe late occurring lung tumours [15, 39]. The decrease of reticulum cell sarcomas mentioned above may also be a phenomenon of competing risks. If one irradiated mice at their thoracic region while shielding the rest of the body, one can induce lung tumours with relatively high observed incidences [2, 7, 27, 30].

Malignant lymphomas in murine thymus are known to develop from lymphocytes present in unirradiated thymus grafted into thymectomized, irradiated mice [18]. All of the induced tumours in Table 2 originated from irradiated tissues and there was no indirect effect in the sense that no tumour was induced in unirradiated tissues.

The tumour incidences given in Table 2 were read with some reservations since the histological examinations were carried out only on tissues with macroscopic abnormalities. Accordingly a few oversights on smaller tumours were unavoidable.

Covelli et al. [5] and Ulrich et al. [28, 29, 32–36] used an age-adjusted incidence of tumours instead of the observed incidence. The reason for this is that the distribution of ages at death differed considerably among the treatment groups. Because of this, they insist that the values for the observed incidence of tumours do not accurately reflect the tumourigenic effectiveness of radiation exposure. Such age-adjusted incidence is a common procedure among
epidemiologists [8]. Thomson et al. [26] used standardized mortality ratios, which are conceptually similar to the age-adjusted incidence. Various methods of correction for the competing risks mentioned above have been applied to the observed incidence by many authors [9, 11, 14, 15, 17]. At present, however, there may be no generally accepted standard method which corrects the observed incidence of experimental radiation tumorigenesis.

REFERENCES

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要　約
X線の部分照射をうけたマウスにおける腫瘍発生：佐藤文昭・佐々木俊作1)・卒野幸利2)・遠藤大二（北海道大学獣医学部獣医放射線学教室、1)放射線医学総合研究所障害基礎研究部、2)国立予防衛生研究所生物制剤管理部）—本実験はX線の部分照射をうけたマウスに見られる発発障害を調べる目的で行った。10週目のddY/ SLC雌マウス42匹を次の4群に分けた。（1）950radの頭部照射群。（2）950radの軸幹部照射群。（3）950radの下肢部照射群。（4）非照射対照群。10週間でX線照射後に終生飼育した。照射後の平均生存期間と標準誤差は以下の通りであった。頭部照射群では430±13日、軸幹部照射群では354±8日、下肢部照射群では435±13日、非照射対照群では472±14日であった。これらのデータから放射線による寿命短縮を計算すると、頭部照射群で9％、軸幹部照射群で25％であった。下肢部照射群の寿命短縮は統計的に有意であった。頭部照射群には下垂体腫瘍が誘発された。軸幹部照射群には卵巣腫瘍が誘発され、悪性リンパ腫の発生が減少した。これらの結果よりX線照射による腫瘍の発生を抑制した。この研究の結果、放射線照射による寿命短縮が大きかったので、この腫瘍の好発年齢まで生存したマウスが少なくなかったためと考えられる。下肢部照射群の腫瘍スペクトルは非照射対照群に比べ、有意の変化は認められなかった。