Tumor Induction in B6C3F1 Mice by Californium-252

TADATERU TAKAHASHI, HIROMITSU WATANABE, YOSHIKI NAKAGAWA, MASAHIRO MORI, HIDEYUKI AYOYAMA and AKIHIRO ITO

Department of Cancer Research, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan.

(Received December 28, 1987)
(Revised version, accepted July 4, 1988)

Californium 252/B6C3F1 mice/Hepatoma

The tumorigenicity of 252Cf, a nuclide being used as a neutron source, was examined in B6C3F1 mice. Both sexes of mice were exposed to 252Cf at different doses of 0 (control), 12.5, 50 or 200 cGy at 6 weeks of age. They were observed for 13 months after exposure. Among the mice exposed to 200 cGy of 252Cf, hepatic tumor was found 55% in male and 29% in female, values being significantly higher than those of the unirradiated controls. Furthermore, the incidence of hepatic tumors increased dose dependently in both sexes (correlation coefficient in males r = 0.72, in females r = 0.98). Number of hepatic tumors and average tumor size correlated to the tumor incidence. Tumor sites were found most frequently in lobe 5 followed by lobe 3. Adrenal tumors in the female mice were observed only in adrenal cortex and also showed dose dependent increase (r = 0.995). Ovarian tumors were found with incidences of 14% in 200 cGy group and 30% in 50 cGy group.

INTRODUCTION

Californium 252 (252Cf) is a spontaneous neutron emitter, decaying by γ-ray emission and spontaneous fission, with half lives for the two processes of 2.6 and 85.5 years, respectively. It is used as a neutron source in radiotherapy. Currently, there is much interest in the biological effects of fission neutron, especially at low dose levels, due largely to the re-evaluation of dosimetry and consequent biological effects of atomic bomb radiation in Hiroshima and Nagasaki. Among the survivors, various neoplasms, including leukemias, thyroid, breast and salivary gland have been found in excess. Recently, increased incidences of stomach and

2. Mice were maintained under the guidelines set forth in the “Guide for the Care and Use of Laboratory Animals” by the Institute of Laboratory Animal Resources, National Research Council, USA.
4. To whom all correspondence should be made.
5. Abbreviation used: B6C3F1 mice; (C57BL/6NxC3H/HeN)F1, 252Cf; Californium-252, RBE: relative biological effectiveness.
lung cancers were also reported\textsuperscript{9}). The contribution of neutrons to those radiogenic cancers, however, is now difficult to assess.

Experimental studies of fission neutron have confirmed induction of neoplasms in rodents\textsuperscript{10-12}). For $^{252}$Cf, there have been studies on the RBE based on mammalian lethality (LD50/30) and effects in cultured cells\textsuperscript{13,14}).

In this study, we have designed experiments to observe carcinogenic effects of $^{252}$Cf in mice with respect to dosage and tumor spectra.

**MATERIALS AND METHODS**

*Animals:* B6C3F\textsubscript{1} mice of both sexes were purchased from Charles River Japan Inc., Kanagawa. Six mice each were housed in autoclaved clean cages with sterilized wood tips, kept in a room with controlled temperature (24 ± 2°C) and humidity. All mice were given diet (MF; Oriental Co. Ltd., Tokyo) and tap water ad libitum.

*Californium 252 Irradiation:* Male and female of 6 weeks old B6C3F\textsubscript{1} mice were irradiated with a Ferris wheel irradiator (Chiyoda Safe Products Co. Japan) at a dose rate of 36 cGy/hr. Individual animals were put into an acrylile mesh container which allowed free movement within the space. The ferris wheel was rotated slowly during irradiation. Absorbed dose of $^{252}$Cf in tissue is composed of 63% fission neutron and 37% $\gamma$-ray. The irradiation times were approximately 21 min for 12.5 cGy, 83 min for 50 cGy and 333 min for 200 cGy, respectively.

*Experimental design:* Mice were divided into following groups: group 1 was male mice and given 200 cGy (group 1-a), 50 cGy (group 1-b), 12.5 cGy (group 1-c) and 0 cGy (group 1-d). Group 2 was female mice and received same doses as male mice (group 2-a, 2-b, 2-c, 2-d). Individual dose groups consisted of 30 mice at the start except in the experiment in groups of 1-d and 2-d (9 males and 17 females).

*Pathology:* All mice were observed every day and weighed once a month. They were observed for 13 months after $^{252}$Cf irradiation. Body and organs were weighed at autopsy, and most organs including those with neoplastic changes were subjected to routine histological studies. Hepatic tumors were stained with either periodic acid shiff (PAS), van-Giesson or immunohistochemical staining for H-ras antigen (Ha-ras antibody was kindly provided by Dr. H. Suku, Nagasaki University, School of Medicine, Nagasaki).

**RESULTS**

*Survival and body weight:* Survival rates at 52 weeks after $^{252}$Cf irradiation were 93% in group 1-a, 100% in group 1-b, 97% in group 1-c and 100% in group 1-d. Similarly they were 93% in group 2-a, 100% in 2-b, 93% in 2-c and 100% in 2-d. The average body and liver weights at the start and end of the experiments are shown in Table 1. In the male, the average body weight in group 1-a was significantly lower than that of control at the end of the experiment.
In the female, however, no significant difference was found in the body weight between group 2-b and control mice (2-d).

### Table 1. Mean body and liver weights

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>Effective no. of mice</th>
<th>Body wt. (g)</th>
<th>Liver wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Start</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1-a</td>
<td>28</td>
<td>25</td>
<td>39 ± 4.0*</td>
</tr>
<tr>
<td></td>
<td>-b</td>
<td>30</td>
<td>24</td>
<td>43 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>-c</td>
<td>29</td>
<td>24</td>
<td>44 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>-d</td>
<td>9</td>
<td>25</td>
<td>43 ± 4.5</td>
</tr>
<tr>
<td>Female</td>
<td>2-a</td>
<td>28</td>
<td>20</td>
<td>38 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>-b</td>
<td>30</td>
<td>20</td>
<td>43 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>-c</td>
<td>29</td>
<td>19</td>
<td>41 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>-d</td>
<td>17</td>
<td>21</td>
<td>38 ± 5.3</td>
</tr>
</tbody>
</table>

a: mean ± SD

*; significantly different from control: p<0.05.

**Tumors:** The incidence of tumors in each group is shown in Table 2. Incidence of total tumors correlated well to the administered doses of $^{252}$Cf. Among the tumors observed, hepatic tumors in both sexes and adrenal tumors in the female showed dose dependent increases. Incidence of ovarian tumors was also high in 50 and 200 cGy irradiated mice.

Hepatic tumors were the highest in incidence among the all observed tumors. Incidence was higher in males (38% in group 1-c) than in females (3% in group 2-c) and increased with doses of $^{252}$Cf in both sexes (correlation coefficient in males $r = 0.72$, in females $r = 0.98$).

Table 3 summarizes the characteristics of hepatic tumors. Histological classification of hepatic tumors, number of hepatic tumors per mouse, and the number of tumors in individual hepatic lobes were examined. Highest localization of the tumor was found in lobe 5 followed by lobe 3. The incidence of hepatic carcinomas in the irradiated males tended to increase with increasing dose of $^{252}$Cf. There were no hepatic tumors metastasized to any other organs.

The tumors were classified histologically into hepatocellular carcinoma and adenoma. Altered foci were not included in the tumor. Most of the tumors consisted of neoplastic cell foci consisting vacuolated cells with degenerative changes. Hepatic carcinomas were composed of more than two liver cell plates, and surrounded by sinusoidal cells. There was also marked cell polymorphism (Fig. 1). Their cytoplasms were also stained with anti-Ha-ras antibody. Cellular arrangement in the hepatic adenomas was smooth and homogenous. The boundaries of adenomas and carcinomas were sharply demarcated from surrounding liver tissue, where cystic hyperplasia of biliary ductules (peliosis hepatis) exist (Fig. 2). In the altered foci, however, no evident edge can be seen and it was almost exclusively composed of vacuolated or glycogen loaded cells.
Table 2. Incidence of tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no. of mice</th>
<th>Total no. of tumor</th>
<th>Hepatic T.</th>
<th>Ovarian T.</th>
<th>Adrenal T.</th>
<th>Pulmonary T.</th>
<th>Lymphoma</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 -a</td>
<td>28</td>
<td>18(62)</td>
<td>16(55)*</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>1(4)</td>
<td>4(14)d</td>
</tr>
<tr>
<td>-b</td>
<td>30</td>
<td>14(47)</td>
<td>13(43)</td>
<td>—</td>
<td>0</td>
<td>4(13)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-c</td>
<td>29</td>
<td>12(41)</td>
<td>11(38)</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>1(3)e</td>
<td>0</td>
</tr>
<tr>
<td>-d</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>1(11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 -a</td>
<td>28</td>
<td>16(57)</td>
<td>8(29)*</td>
<td>4(14)**</td>
<td>6(21)*</td>
<td>1(4)</td>
<td>0</td>
<td>6(21)f</td>
</tr>
<tr>
<td>-b</td>
<td>30</td>
<td>13(43)</td>
<td>2(7)</td>
<td>9(30)**</td>
<td>2(7)</td>
<td>0</td>
<td>0</td>
<td>3(10)g</td>
</tr>
<tr>
<td>-c</td>
<td>29</td>
<td>3(10)</td>
<td>1(3)</td>
<td>0</td>
<td>1(3)</td>
<td>1(3)</td>
<td>1(3)</td>
<td>0</td>
</tr>
<tr>
<td>-d</td>
<td>17</td>
<td>3(18)</td>
<td>1(6)</td>
<td>0</td>
<td>1(6)</td>
<td>0</td>
<td>1(6)b</td>
<td>0</td>
</tr>
</tbody>
</table>

a: Hepatic tumors include adenomas and carcinomas.
b: Ovarian tumors were all sex cord tumors.
c: All tumors were pulmonary adenoma and one adenocarcinoma in group 1-b.
d: One lymphoma, 1 tubular adenoma of the kidney, 1 gastric tumor, 1 fibrosarcoma and 1 papilloma of the skin.
e: One skin papilloma.
f: One pituitary adenoma, 1 gastric adenoma, 1 bladder carcinoma, 1 subcutaneous fibrosarcoma, 2 Hardelian gl. tumor.
g: Two Hardelian gl. tumor, 1 skin papilloma.
h: One pituitary adenoma.
*: Significantly different from respective control by p<0.01.
**: Significantly different from respective control by p<0.05.
Correlation coefficient; hepatic T. in males r = 0.72, y = 0.18x + 21.9
hepatic T. in females r = 0.98, y = 0.13x + 2.9
adrenal T. in females r = 0.995, y = 0.1x + 1.16

Table 3. Incidence of hepatocellular tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>No. of mice with tumor (%)</th>
<th>Tumor no. in each lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ca.</td>
<td>Ad.</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>1 -a</td>
<td>28</td>
<td>16(55)</td>
<td>4(14)</td>
</tr>
<tr>
<td>-b</td>
<td>30</td>
<td>13(43)</td>
<td>3(10)</td>
</tr>
<tr>
<td>-c</td>
<td>29</td>
<td>11(38)</td>
<td>2(7)</td>
</tr>
<tr>
<td>-d</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 -a</td>
<td>28</td>
<td>8(29)</td>
<td>1(4)</td>
</tr>
<tr>
<td>-b</td>
<td>30</td>
<td>2(7)</td>
<td>0</td>
</tr>
<tr>
<td>-c</td>
<td>29</td>
<td>1(3)</td>
<td>0</td>
</tr>
<tr>
<td>-d</td>
<td>17</td>
<td>1(1)</td>
<td>0</td>
</tr>
</tbody>
</table>

a: mean ± SD
Fig. 1. Hepatocellular carcinoma of a male mouse found 13 months after irradiation with 200 cGy of \(^{252}\text{Cf}\). H.E. ×198.

Fig. 2. Hepatocellular adenoma of a mouse found 13 months after irradiation with 200 cGy of \(^{252}\text{Cf}\). H.E. ×75.
Fig. 3. Granulosa cell tumor in the ovary of a mouse found 13 months after irradiation with 200 cGy of $^{252}$Cf. H.E. $\times 100$.

Fig. 4. Adrenocortical tumor in the adrenal of a mouse found 13 months after irradiation with 50 cGy of $^{252}$Cf. H.E. $\times 100$. 
Ovarian tumors were the second highest in frequency. Their incidence was increased in irradiated groups 2-a (14%) and 2-b (30%). Occurrence of the ovarian tumor was usually symmetrical. They were either cystic or solid and yellowish gray in color. Histologically, they could be classified as granulosa cell, granulosa-lutein cell or lutein cell tumors (Fig. 3).

The incidence of adrenal tumors in the female also increased with increasing $^{252}$Cf dose ($r = 0.95$). All were diagnosed as adrenocortical tumors having steroid containing clear cytoplasm (Fig. 4).

Other tumors: Pulmonary tumors, non-thymic lymphomas, pituitary tumors, hardelian gland tumors and soft tissue tumors were often observed.

**DISCUSSION**

In a preliminary study, LD/50/30 of $^{252}$Cf was estimated to be above 390 cGy in B6C3F1 mice and principal damage was observed in the intestinal tract and hematopoietic tissues. In the present study, doses of 200 cGy gave rise to slight retardation of the body growth in both sexes of mice, but no prominent damage (acute or chronic) was detected in the intestinal tract and hematopoietic tissues.

The study clearly showed that $^{252}$Cf irradiation at the three different doses of 12.5, 50 and 200 cGy increased the incidence and multiplicity of major tumors in both sexes of mice. The incidence of hepatocellular carcinomas in the irradiated male tended to increase with increasing doses, although the difference between the control group was statistically not significant. It is well known that the frequency of spontaneously occurring hepatic tumors in B6C3F1 mice is quite high amounting to as much as 21.9% in males and 3.9% in females in a period of 114 weeks observation, 33.2% in males and 1.6% in females for a much longer period of 166 weeks, and moreover that they are susceptible to promotion by chemicals. In this respect, the present results suggest that the administration of $^{252}$Cf has worked as an accelerating agent for hepatocellular tumorigenesis, in which endogenous retrovirus-related sequences may be important in this particular mouse strain. Induction of liver neoplasms was reported in CBA/Cne and B6C3F1 mice following x-ray irradiation, but no dose dependent increase was noted in their incidences in either strains of mouse. In the female, incidence of the ovarian tumor increased significantly at 50 cGy compared to that of control. Although there is little evidence of ovarian tumorigenesis by fission neutron irradiation, a single exposure to high LET radiation will result in a maximal incidence at 1 to 5 Gy and thereafter with plateau. In contrast to the relatively weak ovarian tumorigenesis by chemicals, potent tumorigenicity of irradiation in the ovary may be due to destruction of ova and follicles, and direct disturbance of the hormonal balance between pituitary gland and ovary. Predominant induction of sex cord tumors in the ovary by irradiation was also contrasted to the occurrence of various histological types after chemicals.

Ovarian tumorigenesis was carefully studied in mice after irradiation with $^{137}$Cs gamma rays at various dose rates and age at exposure. The incidence increased with increasing dose rate and
decreased with advancement of age at the time of irradiation. The results of large scale experiments conducted at Oak Ridge National Laboratory showed that incidence of myeloid leukemia and thymic lymphoma increased with dose and dose rate of gamma-rays. Neutrons were several times more leukemogenic than gamma rays at low dose rates. No increase of hepatic tumor incidence was reported.

Induction of adrenocortical tumor in mice has been best achieved by gonadectomy combined with treatment by chemical carcinogens, but irradiation has been known to be least effective for the induction of this tumor. Since corticosteroid producing cells were seen to both adrenocortical and ovarian tumors, common mechanisms might exist in the development of these two tumors.

ACKNOWLEDGEMENT

The study was supported in part by grant-in-aid from the Ministry of Education, Science and Culture. The authors are greatly indebted to Drs. K. Yokoro and S. Sawada for their encouragement and discussions of this study.

REFERENCES


