Tumor Induction by Monoenergetic Neutrons in B6C3F1 mice

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Monoenergetic neutrons/Tumor induction/Mouse.

This study was undertaken to investigate induction of tumors by monoenergetic neutrons in B6C3F1 mice. Individual groups of 6 week-old animals of both sexes (about 30 mice/group) were exposed to 0.5 Gy of various monoenergetic neutrons (dose rate 0.5 cGy/min) and then observed for 13 months. The incidences of tumors (mainly liver neoplasms) in non-irradiated male and female controls were 11% and 0%, respectively. In the irradiated animals, the incidences were 53%, 50%, 60% and 43% in males, and 75%, 81%, 71%, and 85% in females, after 0.18, 0.32, 0.6 and 1.0 MeV neutron exposure, respectively. There were no significant differences in the tumor induction rate among the different energy groups.

INTRODUCTION

The biological effects of monoenergetic neutrons are of clear interest to basic science and radiation protection, as evidenced by a number of reports of *in vitro* studies.[1–11] To our knowledge, however, there has been relatively little work on the genetic effects of monoenergetic neutrons at various energy levels *in vivo*. In order to study the radiobiological effects of neutrons, the Hiroshima University Radiobiological Research Accelerator (HIRRAC) can be operated under conditions of high proton beam currents of 1mA and acceleration voltages up to 3 MeV. Neutron irradiation is possible in the energy range from 0.07 to 1.13 MeV using a lithium target.[12,13]

Specifications for biological irradiation cover monoenergetic beam conditions, dose rates and deposited energy spectra. High dose rates of monoenergetic neutron fields are useful for studying the neutron energy dependence of biological effects, and also the basic mechanisms of action of neutrons. Monoenergetic neutrons which have a narrow neutron spectrum are particularly useful in this regard. Therefore the present study was undertaken to investigate their long-term biological effects in mice, with the focus on tumor development.

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MATERIALS AND METHODS

Animals

Crj:B6C3F1 mice of both sexes were purchased from Charles River Japan Inc. (Hino, Japan) and housed about five per autoclaved cage on sterilized wood chips, in a room with controlled temperature (24 ± 2°C) and humidity (55 ± 10%) under a regular 12-h light, 12-h dark cycle. The animals were maintained according to the 'Guide for Care and Use of Laboratory Animals' established by Hiroshima University. All mice received a normal diet MF (Oriental Yeast Co. Ltd., Tokyo) and tap water *ad libitum*. The experimental protocol was reviewed and approved by the Animal Use Committee at Hiroshima University.

Monoenergetic neutron irradiation

Various energy neutrons were produced in the Hiroshima University Radiobiological Research Accelerator (HIRRAC) with the $^7Li(p,n)^7Be$ reaction. Doses of neutrons and gamma rays were measured at room temperature using paired ionization chambers, IC-17 ATW (FWT Inc. Goleta, CA, USA) or IC-17G (model GM539, FWT Inc. Goleta, CA, USA). The incident neutron energy was calculated by incident proton energy with quantum theory. The incident proton energies were 2.05, 2.2, 2.5 and 2.9 MeV, respectively, then primary energy of produced neutron toward to 30 degrees were 0.254, 0.418, 727 and 1.127 MeV, respectively. According to Lee's theory,[14] the produced neutron energy distribution with thick lithium target can be calculated. Fig. 1 shows the theoretical neutron energy distributions used in following experiments.

Contamination with gamma-rays was less than 3% in the neutron spectrum when using 10 μm-thick ^7Li targets. Indi-
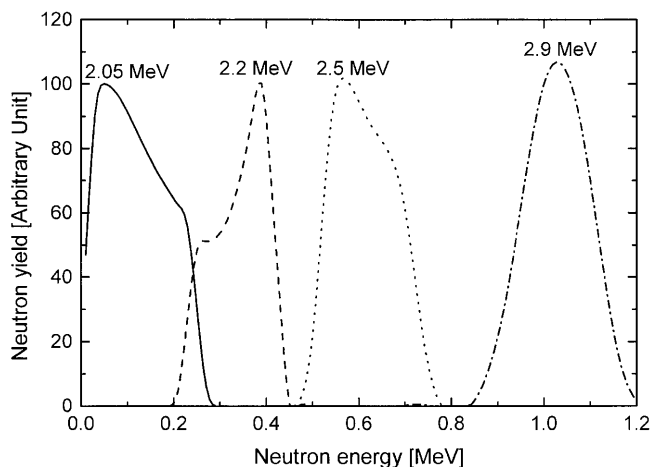
Fig. 1. The neutron energy distribution toward to 30 degrees respect to incident proton beam direction. The distributions are normalized at maximum yield. The energy levels shown on the top of each curve indicate incidence photon energies.
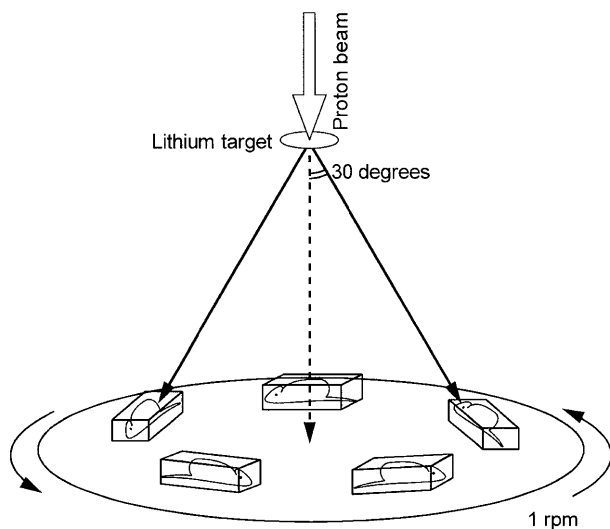

Fig. 2. Experimental setup for neutron irradiation. Five mice were located 20 cm from the neutron producing source at an angle of 30 degrees. In order to provide uniform individual neutron doses, the mice were rotated at a speed of 1 rpm.

vidual mice were placed in individual boxes (3cm × 3cm × 5cm) and groups of 5 mice were located 20 cm from the neutron source at an angle of 30 degrees (Fig. 2) and exposed to monoernergetic neutrons at energy levels of 0.18, 0.32, 0.6 or 1.0 MeV (dose 50cGy, dose rate 0.5cGy/min) without anesthesia. In order to provide uniform individual neutron doses, mice were rotated at a speed of 1 rpm.

Pathology

All animals were regularly observed on a daily basis and weighed once a month. At the time of necropsy, full autopsies were carried out after over ether anesthesia, and body and liver, kidney, adrenal, spleen, testis (male), ovary (female) and uterus (female) weights were determined. The numbers and sizes of liver tumor nodules were also determined and samples of liver and other organs with neoplastic changes were taken and routinely processed for histological examination.

Statistical analysis

The significance of differences in numerical data was determined using 2 × 7 contingency table analysis and the Dunnett's method for multiple comparisons using logarithmic transformation.

RESULTS

Males

Three animals (293, 367 and 362 days after irradiation) died before scheduled autopsy in the 0.18 MeV group, one (365 days) in the 0.32 MeV group, one (319 days) in the 0.6 MeV group and one (403 days) in the 1.0 MeV group. Mean survival period did not significantly differ among the groups. Body weights in the 0.32 MeV group were significantly decreased as compared to control and 1.0 MeV groups. Liver weights with 0.18 MeV were significantly increased as compared with those in control and 0.6 MeV group values, and relative liver weights in the 0.18 MeV group and kidney weights in 0.32 MeV group were elevated (data not shown). There were no other differences in kidney, testis, adrenal and spleen weights among the groups.

Tumor bearing animals accounted for 43% to 60% of the total animals, and the numbers of tumors per animal varied from 0.43 to 0.70. Liver tumors predominated at incidences of 17 to 33%. The number and sizes of liver tumors in the 0.60 MeV group were significantly increased as compared with those in non-irradiated controls but a tendency for decrease was noted with the 1.0 MeV group (see Table 1). There were no significant differences among the irradiated groups. Tumors in sites other than the liver were significantly more frequent in the 0.60 MeV and 1.0 MeV groups than in the controls. Histological findings are summarized in Table 2. Most tumors were rather low in malignancy. However, one osteosarcoma each appeared in the 0.18 MeV and 0.32 MeV groups, one hepatocarcinoma at 0.18 MeV, and 1, 2, and 2 lung adenocarcinomas at 0.18 MeV, 0.32 MeV and 0.6 MeV, respectively. Numbers of hepatomas did not significantly differ among the irradiated groups (Table 2).

Females

Four animals (289, 342, 366 and 388 days after irradiation) died before scheduled autopsy in the 0.18 MeV group, four (219, 276, 366 and 388 days) in the group 0.32 MeV group, three (337, 391 and 419 days) in the 0.6 MeV group, and five (165, 341, 380, 384 and 402 days) in the 1.0 MeV group. There were no differences in the mean survival and

Table 1. Mean survival and tumor induction in male mice

Group	Effective animal	Mean survival (Days)	Tumor bearing animals (%)	No of tumor /mouse	Liver Incidence (%)	No / mouse	Size (mm)	Lung (%)	Other (%)
0.18 MeV	30	407 ± 25	16(53)*	0.53 ± 0.50**	10(33)*	0.43 ± 0.73	3.33±6.56	4(13)	2(7)
0.32 MeV	26	412 ± 10	13(50)*	0.54 ± 0.58**	8(31)*	0.38 ± 0.64	3.21±3.52	3(12)	3(12)
0.60 MeV	30	410 ± 6	18(60)*	0.70 ± 0.75**	12(40)*	0.57 ± 0.82**,a	4.07±7.28*	2(7)	8(27)*
1.0 Mev	30	414 ± 6	13(43)*	0.43 ± 0.50*	5(17)*	0.20 ± 0.48a	0.95±2.93	2(7)	6(20)*
Control	36	413 ± 2	4(11)	0.11 ± 0.32	4(11)	0.11 ± 0.32	0.81±2.64	0	0

Mean ± SD
*: Significantly different from Control value (P < 0.05)
**: Significantly different from Control value (P < 0.01)
a :Significantly different between 0.6 MeV and 1.0 MeV(P < 0.05)

Table 2. Numbers of histological typing tumors of male mice

Group (Mev)	Liver Hepatoma	Hepatocelluar - carcinoma	Lung Adenoma	Adeno - carcinoma	Other No	Tumor type
0.18	12	1	3	1	2	Harderian gland adenoma 1 Bone osteosarcoma 1
0.32	10	0	1	2	3	Harderian gland adenoma 1 Bone osteosarcoma 1 Skin papilloma 1
0.6	17	0	0	2	8	Harderian gland adenoma 2 Pituitary adenocarcinoma 2 Leukemia 1 Muscle sarcoma 2 Adrenal cortical adenoma 1
1.0	6	0	2	0	6	Harderian gland adenoma 3 Pituitary adenoma1 Skin papilloma 2
Control	5	0	0	0	0	

body weights among the female groups. Uterus weights in all irradiated groups were significantly decreased as compared to the control values, whereas adrenal weights increased in 0.18 MeV group (relative to both control and 1.0 MeV groups). Relative liver weights at 0.32 and 0.6 MeV and kidney weights at 0.32 MeV were significantly higher than control values while weights of adrenals at 1.0 MeV and spleen at 0.6 Mev were significantly decreased as compared to the 0.18 MeV values (data not shown).

Tumors were found in 71% to 85% of animals in the irradiated groups, with mean incidences of 1.27 to 1.56 tumors per animal (see Table 3). The highest incidences were noted for tumors of the ovary (41–54%), followed by the Harderian glands. There were no significant differences among the radiation groups. From histopathological findings, radiation-induced tumors were rather low in malignancy (Table 4). However, 3, 3, 1, and 6 malignant granulosa cell tumors in the ovary were observed at 0.18, 0.32, 0.6 and 1.0 MeV, respectively, and three had metastasized to the lungs in the 1.0 MeV group.

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Table 3. Mean survival and tumor induction in female mice

	Effective No	Mean survival (Days)	Total (%)	No of tumor / mouse	Ovary (%)	Lung (%)	Liver (%)	Harderian Gland (%)	Lymphoma (%)	Others (%)
0.18 MeV	28	412 ± 35	22(79)*	1.32 ± 0.47*	19(68)*	1(4)	4(14)	2(7)	2(7)	2(7)
0.32 MeV	27	410 ± 49	22(81)*	1.27 ± 0.55*	18(67)*	2(7)	1(4)	3(11)	1(4)	4(15)
0.6 MeV	28	421 ± 17	23(82)*	1.38 ± 0.57*	15(54)*	3(11)	4(14)	7(25)*	0	6(21)*
1.0 MeV	27	408 ± 52	25(93)*	1.56 ± 0.83*	15(56)*	1(4)	7(26)	6(22)*	2(7)	2(7)
Control	36	427 ± 1	0	0	0	0	0	0	0	0

Mean ± SD
*: Significantly different from Control value (P < 0.05)

Table 4. Number of histological typing tumors in male mice

Group (MeV)	Ovary			Lung		Liver		Harderian gland		Others	
	Tubulostomal adenoma	Benign granulosa cell tumor	Malignat granulosa cell tumor	Adenoma	Adeno-carcinoma	Hepatoma	Hepatocellular-carcinoma	Adenoma	Adeno-carcinoma	No	
0.18	13	3	3	0	1	3	0	2	0	2	Adrenal cortical adenoma 1
											Lymphoma 1
0.32	10	5	3	0	1	2	1	2	1	4	Muscle sarcoma 1
											Pituitary adenoma 3
0.6	9	5	1	1	0	3	1	5	2	6	Pituitary adenoma 1
											Uterus endometrial tumor 1
											Muscle sarcoma 1
											Skin papilloma 1
											Adrenal cortical adenoma 2
1.0	6	6	6	1	0	4	3	5	1	2	Skin squamous cell carcinoma 1
											Mammary adenocarcinoma 1
Control	0	0	0	0	0	0	0	0	0	0	

DISCUSSION

In the present study, there were no consistent differences in tumor incidence among the groups with various energies of neutron irradiation. In males, the incidence, number and size of liver tumors were significantly increased in irradiated animals as compared with non-irradiated controls, but without significantly differences among the irradiated groups. In females, some tumors in the irradiated groups were significantly more common than in their non-irradiated counterparts but again there was no significant variation with the dose applied. Induced tumor incidences in males were 43%–60% and in females were 79%–93%. Takahashi *et al*[15] reported an incidence of 46.7% (hepatic tumors 43.3%) in B6C3F1 male mice and 23.3% in females receiving 50 cGy of ^{252}Cf neutrons, while we[16] reported tumor incidences of

30% in both sexes of mice receiving 42.5 cGy of 290 MeV/u carbon-iron irradiation. In males, the tumor incidence seems to be the same between ^{252}Cf neutrons and monoenergetic neutrons but the incidence of tumors after heavy-iron irradiation was less than that after neutrons in the present experiment. In females, the tumor incidence in the present experiment was higher than with ^{252}Cf neutrons.

Inverse dose-rate dependence of fission-spectrum neutron induction on somatic *hprt* mutations in mouse leukemia L5178Y cells has been reported by Nakamura and Sawada[10], Hill *et al* observed that reduction of the dose rate of fission neutrons increased their effectiveness for transformation of C3H 10T1/2 cells.[17,18] Brenner and Hall published a model of an inverse dose-rate effect for neoplastic transformation *in vitro* following high LET irradiation.[19] Harrison and Bakcer-Kubiczek *et al*, however, found modification of fission neutron dose-response curves by dose rate

to be negligible or absent.[20, 21)] Watanabe *et al* reported that a single ^{252}Cf neutron dose resulted in higher incidences of ovarian and Harderian gland tumors than the same total dose given at a low dose rate with B6C3F1 mouse whole body irradiation.[22)] Clearly there may be differences in dose-rate effect between the *in vitro* and *in vivo*. It is considered that cells with large chromosomal aberrations or other abnormalities might be able to survive *in vitro*, but *in vivo* they might not, so smaller non-lethal chromosomal changes such as point mutations, frame shifts, as small insertions or deletions could be essential for tumor induction *in vivo*. The source of irradiation, strain, sex, and age are all clearly the factors, which need to be taken into account when determining radiation sensitivity. The reason why tumor incidences in mice were not influenced by the various neutron energies is not understood; however, Sasaki *et al*[23)] recently reported that induction of chromosome aberrations is not largely dependent on neutron energy. Further studies on different biologic endpoints are required to address this issue.

In conclusion, there were no consistent differences in tumor incidence among the various energies of neutron irradiation applied.

ACKNOWLEDGEMENTS

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