J. RADIAT. RES., 39, 93–100 (1998)

Comparison of Tumorigenesis between Accelerated Heavy Ion and X-ray in B6C3F1 Mice

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(*Received, July 7, 1997*)
(*Revision received, December 8, 1997*)
(*Accepted, February 9, 1998*)

Heavy ion/X-ray/Whole body irradiation/Tumorigenesis/Mice

The effects of heavy ion and X-ray irradiation on tumorigenesis in B6C3F1 mice were compared. Six-week-old animals were divided into 6 groups and exposed to 0.426 Gy heavy ion irradiation of 290 MeV/u carbon-ion beam (LET 60–210 KeV/μm) at the dose rate of 0.4 ± 0.2 Gy/min; 0.5 Gy of X-ray irradiation at 0.1 Gy/min or 5 Gy of X-ray irradiation at 1 Gy/min. The mice were killed and an autopsy performed 13.5 months after the whole body irradiation. Body weights were heaviest for both sexes in the 0.5 Gy group and lightest in the 5 Gy one. Total tumor incidences in the males were 30, 56 and 13% respectively in the heavy ion, 5 Gy and 0.5 Gy X-irradiated groups, stomach tumors, lymphomas and adrenal tumors being the most common outcome of the high dose X-rays. Liver tumor induction did not differ significantly among the groups. In the females tumorigenicity was significantly lower for heavy ion than for 0.5 Gy and 5 Gy X-ray irradiation ($P < 0.05$), the respective incidences, mainly ovary one, being 73%, 17% and 41%. Non-cancerous lesions, such as graying of the hair, glomerular sclerosis and amyloidosis appeared in the 5 Gy group. These findings indicate that 0.426 Gy of heavy ion irradiation induced lower carcinogenicity than 5 Gy of X-irradiation and higher carcinogenicity than that of 0.5 Gy X-irradiation in male mice.

INTRODUCTION

Radiation carcinogenesis has been studied at the molecular, cellular, and whole-body level[1,2]. A large study of the potential therapeutical effects of heavy ion beams has been started at the National Institute of the Radiological Science (NIRC), and Heavy Ion Medical Accelerator in Chiba (HIMAC); therefore, detailed understanding of any toxicological or carcinogenic influences is needed. Although only few studies have been done, both carcinogenesis and degenerative

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lesions are reported[3–6]. Elsewhere we reported that the average relative biological effectiveness (RBE) of ^{252}Cf neutrons versus ^{60}Co-γrays in mouse liver tumorigenesis is 15.2 in males and 2.5 in females[7] accompanied by the induction of ovarian tumors. There is, however, no available RBE data on heavy ion beam tumorigenesis in mice. Our study therefore was conducted to compare the effects of heavy ion beams with those of X-rays.

MATERIALS AND METHODS

Six-week-old male and female B6C3F1 mice (C57BL/6NCrj × C3H/HeNCrj) purchased from Charles River Japan, Inc. (Hino, Japan) were housed in autoclaved cages in wood chips in a room with controlled temperature (24 ± 2°C) and humidity (55 ± 10%) and regular 12-h light, 12-h dark cycle. The animals were divided into 2 lots, one being sent to NIRS, Chiba, the other to the Research Institute for Radiation Biology and Medicine (RIRBM), Hiroshima University, Hiroshima. One month after irradiation, the animals were shipped from Chiba to Hiroshima by air. All the animals were maintained under the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" established by the NIRS and Hiroshima University. All were fed a normal MF diet (Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water *ad libitum*.

The mice were divided into 6 approximately equal groups. Groups 1 to 3 consisted of males and groups 4 to 6 females. Groups 1 and 4 were irradiated with 5 Gy of X-rays groups, 2 and 5 with 0.426 Gy of heavy ion beams, and groups 3 and 6 with 0.5 Gy of X-rays. The irradiation was whole body without anesthesia.

Accelerated carbon mono peak beams of a 290 MeV/u carbon-ion beam were generated at the HIMAC facility (LET 60–210 KeV/μm) in the NIRC and heavy ions were directed 2 cm from the upper distal end of the spread out beams (SOBP) at the dose rate of 0.4 ± 0.2 Gy per minute for a total dose of 0.462 Gy to the animal surface[8]. X-irradiation was done in the RIRBM, Hiroshima University. Exposure factors were 250 kVp, 25 mA, no filter, half-value layer 1.18 mm Cu, and the dose rate in air 0.1 Gy or 1 Gy per minute.

All the treated mice were observed daily and weighed once a month. They were killed 13.5 months after irradiation and an autopsy performed under ether at which time the body and major organ weights were measured. The excised tissues were fixed in 10% neutral formalin and sections routinely prepared and stained with hematoxylin and eosin for histological evaluation. Lesions in the livers and other affected organs, including neoplastic changes, were diagnosed as described elsewhere[7].

RESULTS

In the 5 Gy X-ray group 3 males and 8 females died within four hundred days of the irradiation. All the other animals were killed and given autopsies between day 406 and 418. Mean survival time for the female 5 Gy group (378 ± 77) was significantly shorter than the times for the male heavy ion (410 ± 0.4 days), and male (411 ± 2 days) and female 0.5 Gy (411 ± 2 days)

groups ($P < 0.05$)

Body weights of the 5 Gy X-irradiated males were significantly decreased as compared to the heavy ion- and 0.5 Gy X-ray-irradiated males throughout the experimental period. (Fig. 1) In

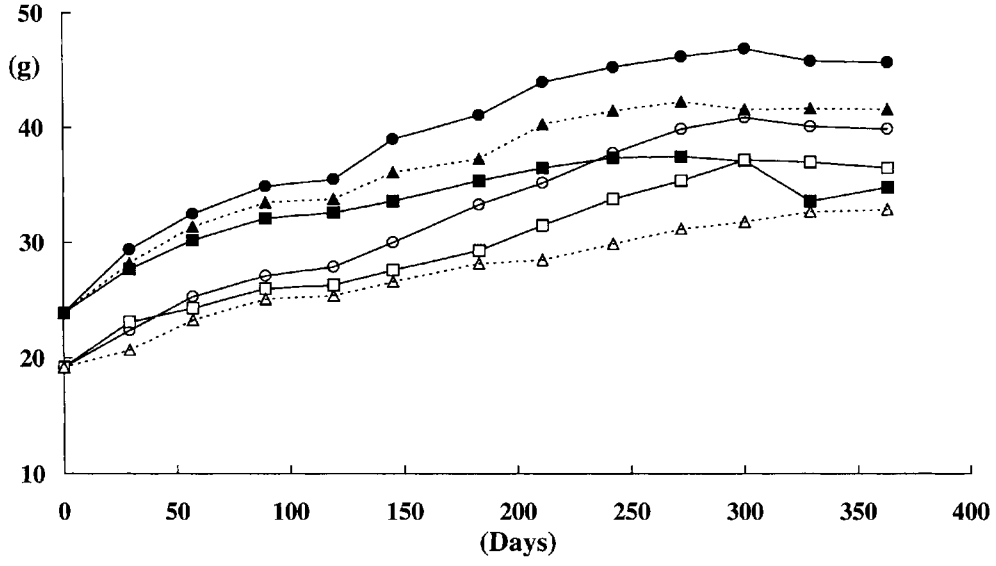

Fig. 1. Change in body weights in B6C3F1 mice after irradiation
■: Male, 5 Gy X-ray, ▲: Male, 0.426 Gy Heavy ion, ●: Male, 0.5 Gy X-ray, □: Female, 5 Gy X-ray, △ : Female, 0.426 Gy Heavy ion, ○: Female, 0.5 Gy X-ray

Table 1. Body and organ weights in B6C3F1 mice after irradiation

Group	No. of mice	Body (g)[a]	Liver (g)[a]	Kidney (mg)[a]	Spleen (mg)[a]	Testis or ovary (mg)[a]	Uterus (mg)[a]	Adrenal (mg)[a]
Male								
X-ray (5 Gy)	32	32.5 ± 5.7	1.77 ± 0.31	547 ± 81	136 ± 82	193 ± 34		20 ± 45
Heavy ion (0.426 Gy)	30	39.7 ± 6.5[b]	1.97 ± 0.41[d]	604 ± 74	111 ± 23	227 ± 38		8 ± 1
X-ray (0.5 Gy)	30	45.8 ± 3.2[b,c]	2.11 ± 0.42[b]	619 ± 64	122 ± 23[c]	247 ± 14[e]		8 ± 2
Female								
X-ray (5 G y)	32	32.0 ± 8.6	1.61 ± 0.54	377 ± 76	192 ± 227	130 ± 356	143 ± 54	21 ± 33
Heavy ion (0.426 Gy)	30	33.6 ± 4.6	1.46 ± 0.21	367 ± 28	145 ± 77	27 ± 16	365 ± 158[b]	10 ± 2
X-ray (0.5 Gy)	30	39.6 ± 5.4[b,c]	1.52 ± 0.23	372 ± 30[e]	207 ± 304	83 ± 228	194 ± 52[b,c]	12 ± 2

[a]mean ± SD
[b]Significantly different from the X-ray (5 Gy) values ($P < 0.01$)
[c]Significantly different from the heavy ion values($P < 0.01$)
[d]Significantly different from the X-ray (5 Gy) values ($P < 0.05$)
[e]Significantly different from the heavy ion values ($P < 0.05$)

the males the liver weights also were significantly decreased (Table 1). Spleen and testis weights in the males and kidney and uterus weights in the females of heavy ion group were decreased as compared to those for the 0.5 Gy groups. Relative weights (organ weight/body weight × 1,000)

Table 2. Relative organ weights in B6C3F1 mice after irradiation

Group	No. of mice	Liver[a]	Kidney[a]	Spleen[a]	Testis or ovary[a]	Uterus[a]	Adrenal[a]
Male							
X-ray (5 Gy)	32	54.1 ± 6.9[b]	16.9 ± 2.4[d]	4.19 ± 2.86[b]	5.85 ± 0.44		0.59 ± 1.29
Heavy ion (0.426Gy)	30	48.8 ± 8.6	15.0 ± 1.2	2.80 ± 0.72	5.64 ± 0.80		0.21 ± 0.06
X-ray (0.5Gy)	30	45.9 ± 5.9[c]	13.5 ± 0.9[c,d]	2.67 ± 0.45[c]	5.42 ± 0.47[c]		0.18 ± 0.04[b]
Female							
X-ray (5 Gy)	32	48.0 ± 20.7	11.4 ± 4.6	5.87 ± 7.82	3.75 ± 10.26	4.25 ± 2.02[d]	0.57 ± 0.86
Heavy ion (0.426 Gy)	30	43.9 ± 7.2	11.0 ± 1.2	4.41 ± 2.64	0.81 ± 0.46	11.06 ± 4.87	0.31 ± 0.08
X-ray (0.5Gy)	30	38.7 ± 4.9[d,e]	9.5 ± 1.0[b,c]	5.19 ± 7.33[b,e]	2.12 ± 5.90	5.02 ± 1.76[d]	0.30 ± 0.09

[a]mean ± SD
[b]Significantly different from heavy ion ($P < 0.05$)
[c]Significantly different from X-ray (5 Gy) ($P < 0.01$)
[d]Significantly different from heavy ion ($P < 0.01$)
[e]Significantly different from X-ray (5 Gy) ($P < 0.05$)

Table 3. Tumor Incidences in B6C3F1 mice after irradiation

Group	No. of mice	Total Tumors	Liver	Stomach	Harderian	Lung	Lymphoma	Ovary	Adrenal	Others
Male										
X-ray (5 Gy)	32	18 (56)	6 (19)	4 (13)	1 (3)	0	3 (9)		3 (9)	papillomas 2 duodenum 1 testis 1 leukemia 1 sarcoma 1
Heavy ion (0.426 Gy)	30	9 (30)	7 (23)	1 (3)	0	1 (3)	0		1 (3)	0
X-ray (0.5 Gy)	30	4 (13)[a]	4 (13)	0	0	1 (3)	0		0	0
Female										
X-ray (5 Gy)	32	22 (69)	4 (13)	1 (3)	5 (16)	0	3 (9)	13 (41)	0	anus 1 sarcoma 1 salivary 1 duodenum 1
Heavy ion (0.426 Gy)	30	9 (30)[a]	1 (3)	3 (10)	3 (10)	1 (3)	0	5 (17)	0	0
X-ray (0.5 Gy)	30	22 (73)	0	0	0	0	2 (7)	22 (73)	0	

[a]Significantly different from the X-ray (5 Gy) value ($P < 0.01$)

of the liver, kidney and spleen in the male heavy ion group were significantly decreased in comparison to those in the 5 Gy group, the testis weights in the 0.5 Gy group significantly decreased as compared to those in the 5 Gy groups and the adrenal gland weights in the heavy ion group were significantly decreased as compared to those in 0.5 Gy group. (Table 2) In the females, the liver, kidney and spleen weights in the heavy ion and 5 Gy groups were significantly increased as compared to those in the 0.5 Gy group. Uterus weights in the heavy ion group were significantly increased as compared to those in the 5 Gy and 0.5 Gy groups.

The first tumor, a thymic lymphoma, appeared 166 days after the 5 Gy irradiation. The total tumor incidence was significantly greater for the 5 Gy group as compared to the 0.5 Gy group ($p < 0.01$) in the males and for the heavy ion group in the females. The tumor spectrum (mainly stomach, lymphnodes, and adrenals in the males and the Harderian glands and lymphnodes in the females) was wider in the 5 Gy group than in the other treated groups

The incidence, sizes, and numbers of liver tumors, however, did not differ significantly. (Tables 3, 4)

In the females, ovarian tumors (mainly granulosa cell lesions) were most common in the 0.5

Table 4. Incidences and numbers of liver tumors in B6C3F1 mice after irradiation

Group	No. of Mice	Tumor incidence (%)	Tumor size (mm)[a]	No. of tumors /mouse[a]
Male				
X-ray (5 Gy)	32	4 (19)	1.06 ± 2.84	0.25 ± 0.51
Heavy ion (0.426 Gy)	30	7 (23)	1.27 ± 3.53	0.30 ± 0.60
X-ray (0.5Gy)	30	4 (13)	1.38 ± 3.76	0.10 ± 0.31
Female				
X-ray (5 Gy)	32	4 (13)	0.50 ± 1.46	0.13 ± 0.33
Heavy ion (0.426 Gy)	30	1 (3)	0.07 ± 0.37	0.03 ± 0.18
X-ray (0.5Gy)	30	0	0	0

[a]mean ± SD

Table 5. Induction of non-cancerous lesions in B6C3F1 mice after irradiation

Group	Fatty liver	Glomerular sclerosis	Amyloidosis (spleen)	Hair graying
Male				
X-ray (5 Gy)	5/32 (16)	9/32 (28)	5/ (16)	32/32 (100)
Heavy ion (0.426 Gy)	5/30 (17)	0/30	0/30	0/30
X-ray (0.5 Gy)	7/30 (23)	0/30	0/30	0/30
Female				
X-ray (5 Gy)	0/32	9/32 (28)	1/32 (3)	32/32 (100)
Heavy ion (0.426 Gy)	0/30	0/30	0/30	0/30
X-ray (0.5 Gy)	0/30	0/30	0/30	0/30

Gy group.

With regard to non-cancerous lesions (Table 5) graying of the hair was observed in both mice sexes from 3 months after 5 Gy irradiation. Glomerular sclerosis and amyloidosis in the spleen also were present in both the males and females of the 5 Gy-irradiated group.

DISCUSSION

The present findings showed intermediate tumorigenicity between 0.5 and 5 Gy of X-rays in the heavy ion irradiation of male B6C3F1 mice. Females appeared to be less susceptible. Previously we reported an RBE of 15.2 for liver tumorigenesis in mice given Cf neutron irradiation[7]. Rodriguez et al[4] found respective RBE values of 1.0, 1.1 and 1.3, for rat 9L spheroids survival of mouse intestinal crypt cells, and mouse testis weight loss for carbon 400 MeV/u irradiation. Kojima et al[5] reported a value of 1.2 for femoral CFU-S exposed to heavy ions (290 MeV/u carbon-12, 1.8 for splenic CFU-S in the terminal resistant portion of the survival curve, and 1.14 for the LD50/30. The RBE for radiation injury to the spinal cord of male rats by heavy ion particles (290 MeV/u carbon-12) is estimated to be approximately 2 (Okeda et al, personal communication). The RBE for heavy ion beams in the males was lower than that for Cf neutrons[7]. In the male mice there was no significant differences in liver tumors between any two groups, but there was a relationship with fatty livers and liver tumors. Grahn et al[9] reported a linear dose response for liver tumor induction in mice for fission neutrons and ^{60}Co γrays. If this is valid the RBE value for heavy ion beam would be higher than that for X-rays.

In female mice the ovary is one of the most radiosensitive organs. Upton et al[10] reported that the dose-incidence relation for gamma-irradiation at high dose rates follows a sigmoid curve, rising from the control level between 25 and 50 cGy, reaching a maximum at 100 to 150 cGy, and decreasing at 300 cGy. Clapp[11] found a 50 to 60% incidence of ovarian tumors in young adult female RF/Un mice given 50 to 100 cGy of X rays in single doses, a gradual decline being found for 100 to 400 cGy. Covelli et al[12] reported similar dose-dependence in intact ovaries and irradiated ovaries transplanted into host animals, a steep rise occurring up to 0.75 Gy followed by a plateau up to 4 Gy. Ullrich et al[13] obtained equivalent results for the neutron irradiation of female BALB/c mice. In our experiment, however, the induction of ovarian tumors by X-rays was not dose dependent, and the response to the heavy ion irradiation was limited. Further investigations are needed of tumorigenesis of the liver in male mice and of the ovary in female mice caused by various doses of heavy ion beams.

In the 5 Gy-irradiated groups glomerular sclerosis and amyloidosis appeared, which agrees with earlier reports that therapeutic abdominal irradiation may be accompanied by late-occurring progressive renal disease associated with glomerular mesangial sclerosis, tubular degeneration, and amyloidosis[14–16]. Jaenke et al[17] described sclerosis with continued reduction of the glomerular filtration rate in kidneys of mature pigs 12 weeks after irradiation with a single dose of 9.8 Gy ^{60}Co gamma-rays. These findings suggest that glomerular sclerosis appears first and that amyloidosis is a secondary change. Our findings suggest that high dose X-ray-treated mice may serve as a new model for the induction of glomerular sclerosis and amyloidosis.

Graying of the hair of the mice appeared 3 months after 5 Gy of X-irradiation, but not in the other groups. Such graying occurs in mice after local X-ray irradiation whereas in rats loss of hair appears three months after local 10 Gy X-irradiation. (Watanabe et al, unpublished data) Malinson and Keane[18] found that doses of 7–8 Gy or more of ionizing radiation induce some degree of permanent hair loss in the pig, and Sieber et al[19] reported that this is linear and single dose-dependent for exposures between 1.0 and 15.0 Gy. The ED50 for the loss of > or = 30% of the hair was 3.8 Gy, whereas that for the loss of > = 50% was 6.8 Gy and for the loss of > or = 67% of 12.5 Gy[20]. These findings suggest that the induction the graying of mouse hair is a high dose phenomenon.

ACKNOWLEDGEMENTS

This study was supported by funds from the Heavy Ion Research Project of the NIRS-HIMAC. We thank the members of the Heavy Ion Research Project at NIRS-HIMAC, and those of the Department of Radiation Research of this Institute for animal X-ray irradiation, as well as Ms. M. Tanizaki for her technical assistance and Ms. Y. Matsui for her secretarial help.

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