Induction of Insulinomas and Kidney Tumors in SD Male Rats by X-Irradiation of Gastric Region

Hiromitsu Watanabe^{1,4}, Midori Tanizaki¹, Nariaki Fujimoto², Ken Haruma³, Yoshiyuki Masaoka¹, Masao Kurosumi¹, Shuneki Shoji¹, and Osamu Katoh¹

¹Department of Environment and Mutation, ²Department of Cancer Research, Research Institute for Radiation Biology and Medicine, ³First Department of Internal Medicine, School of Medicine, Hiroshima University

Abstract: X-ray induction of tumors were examined in five-week-old male CD(SD): Crj rats, treated with two 10 Gy doses to the gastric region with a 3-day interval (total 20 Gy). After irradiation, the rats received a 1% NaCl supplement in the diet and survivors were killed 22 months after the first X-irradiation. The total tumor incidence was 59% (48 of 82 effected rats), the first animal demonstrating a sarcoma at day 273. Islet tumors of the pancreas appeared from the 455 day time point. Pancreatic and kidney tumors developed in 15 rats (18%) each, and apocrine tumors at the irradiated site and sarcomas in 9 rats (11%) and 5 (6%), respectively. Gastric tumors were found in 3 rats (4%). Immunohistochemistry revealed the islet tumor cells to be generally positive for insulin, although some cells contained somatostatin or glucagon, but not gastrin. The present results thus showed that the insulinomas and tumors other than gastric lesions are induced by X-irradiation of the upper peritoneal region. (*J Toxicol Pathol* 1997; 10: 77~81)

Key words: X-irradiation, Insulinoma, Kidney tumor, Rat

Introduction

Although experimental induction of intestinal metaplasia in the glandular stomach of rats by X-irradiation has been repeatedly reported¹⁻⁶, its relevance to gastric neoplasia remains unclear. While a few tumors were observed³ and their numbers were increased by application of higher split doses⁵⁻⁷. The studies were of limited duration and observation was for no longer than 1 year after X-irradiation. The present investigation was performed to assess lesion development in the long term. We have described our findings for induction of pancreatic, kidney, and other tumors by X-irradiation.

Materials and Methods

Animals

Eighty-three five-week-old male Crj: CD(SD) rats (Charles River Japan Inc., Hino) were used in the present study. They were housed three of four to a polycarbonate cage and kept under constant conditions of temperature ($24 \pm 2^\circ\text{C}$), and relative humidity ($55 \pm 10\%$) with a 12 h light/12 h dark cycle. The animals were maintained under the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University.

X-irradiation

Rats were anesthetized with Nembutal and X-irradiated according to the method described previously¹⁻⁴. A 0.6-cm-thick lead cover, with a hole 1.8 cm in diameter, was positioned so that the hole lay over the gastric region. Each animal was given two X-ray doses of 10 Gy each with a three-day interval (total 20 Gy). Exposure factors were as follows: 200 kVp, filter 0.5 Cu + 1.0 Al, half-value layer 1.18 mm Cu, at a dose rate of 90 R/min as measured with a Radicon 555 decimeter. The X-ray air dose (in R) was then converted to the absorbed dose (in cGy) using a factor of 0.95 cGy/R.

Examination of animals

All animals were regularly observed and the survivors were killed sequentially or at the termination of the experiment after 22 months. The stomachs were cut open along the greater curvature, stretched out and pinned on a board with the mucosal surface facing upward. Each stomach was washed several times with physiological saline before gross examination, and fixed in 10% neutral formalin. Alkaline phosphatase (ALP) activity in the gastric mucosa was determined by the naphthol-AS-MX-phosphate-fast blue RR staining method⁸. The numbers of crypts with ALP activity in both the pylorus and fundus were counted using a dissecting microscope. Strips of the stomach were cut perpendicularly to the mucosal surface, two strips being taken through the lesser curvature and four through the greater curvature. The strips were embedded in paraffin and serially sectioned at 3 μm . Sections were routinely stained with hematoxylin and eosin, and for clarification when necessary the periodic acid-Schiff (PAS)-alcian blue and high-iron diamine (HID)-alcian blue (AB) staining procedures were adapted. Gastric lesions in the glandular stomach were classified into atypical hyperplasia and adenocarcinoma categories. The

渡辺敦光 谷崎みどり 藤本成明 春間 賢 正岡良之 黒住正雄 荏司俊益 加藤 修

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Mailing address: Hiromitsu Watanabe, Department of Environment and Mutation, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan.

Table 1. Body and Organ Weights

Total	Body (g)	Liver (g)	Kidney (g)	Testis (g)	Spleen (g)	Adrenal (mg)
No. of animals	82	56	55	54	53	55
Weight	649±109	17.9±3.7	6.21±8.49	3.48±0.36 ^{a)}	0.961±0.939	98.7±84.2
Relative weight		27.6±5.5	10.04±14.76	5.36±0.82	1.51±1.68	0.154±0.129
400-499 days						
No. of animals	48	35	35	35	35	35
Weight	654±102	17.7±2.5	4.61±2.33	3.52±0.33 ^{a)}	0.882±0.239	102.7±104.2
Relative weight		26.9±4.3	7.24±5.06	5.38±0.79	1.35±0.46	0.157±0.156
500-599 days						
No. of animals	19	12	12	11	12	12
Weight	683±114	18.6±6.1	4.85±1.48	3.64±0.22 ^{a)}	1.315±1.956	79.8±15.3 ^{a)}
Relative weight		27.8±6.6	7.29±1.87	5.41±0.99	2.13±3.50	0.124±0.038 ^{b)}
over 600 days						
No. of animals	9	8	8	8	6	8
Weight	628±98	17.9±4.4	15.6±20.4	3.10±0.42	0.768±0.367	115.6±0.034
Relative weight		29.6±8.5	26.7±34.3	5.09±0.79	1.25±0.53	0.195±0.074

^{a)}: Significantly different from the over 600 day value ($P<0.01$)

^{b)}: Significantly different from the over 600 day value ($P<0.05$)

numbers of intestinal metaplastic crypts on the same slide were counted separately for both the pyloric glands and the fundic glands. Metaplastic crypts within 5 crypts from the pyloric ring were not scored.

The immunoglobulin enzyme bridge technique (indirect method) was employed for immunohistochemical studies. Dewaxed formalin-embedded tissue were cut and sections were incubated for 30 min at room temperature with rabbit anti gastrin, glucagon, insulin, and somatostatin antibodies (Shandon-Lipshaw). Then the sections were sequentially incubated with a biotin-labeled secondary antibody and an alkaline phosphatase conjugated streptavidine complex using new fuchsin as the chromogen substrate all purchased form (DAKO LSAB kit alkaline phosphatase system 40, K0628, DAKO).

Fig. 1. Animal survival and the cumulative tumor incidence after X-irradiation.

■: Animal survival; ○: Tumor incidence

Statistical analysis

The significance of differences in numerical data was evaluated using the chi-squared test, the Student's *t* test and by fitting calibration lines using a linear equation.

Results

Eighty-two rats which survived beyond 266 days after the start of X-irradiation were counted in the effective number, those dying before this time point being without

Fig. 2. Cumulative incidences of various types of tumor after X-irradiation.

—○—: Gastric tumor; —■—: Pancreatic tumor; ---: Kidney tumor; - - - - -: Apocrine tumor; —: Sarcoma in abdominal; —○—: Sarcoma in skin; —*—: Bile duct tumor; —: Other tumor

tumors. Six animals were killed or dead at 399 days, 10 animals at 449, 38 animals at 499, 16 animals at 549 days, 3 animals at 599 days, and 9 animals after 600 days. (**Table 1**, Fig. 1)

Data for mean body and organ weights at the various time points are summarized in Table 1. Body weights quickly increased until day 114 after X-irradiation, gradually rose until day 546 and decreased thereafter. Kidney weights increased after day 600 in the surviving group while testis weights were decreased.

Curves for animals survival and cumulative tumor incidences are shown in Figs. 1, 2. A sarcoma of the skin, the first tumor, appeared on day 273 after the first X-irradiation and a gastric tumor was noted on day 510. Kidney tumors (Fig. 3) were the next type to be followed by pancreatic (Figs.

4, 5) and finally bile duct tumors. The total tumor incidences was 58.5%, pancreatic and kidney epithelial tumors being found in 15 animals each (18%), apocrine tumors (Fig. 6) and abdominal sarcomas in 9 each (11%), skin sarcomas in 5 (6%), bile duct and well-differentiated small intestinal tumors in 2 (2%), and liver and lung tumors in 1 (1%) (**Table 2**).

The pancreatic lesions were diagnosed as insulinomas, binding anti-insulin antiserum (Fig. 5), with tumors being also positive for somatostatin and glucagon but not gastrin.

ALP-positive metaplastic foci in both the pylorus and fundus were frequently found, the average number being 4.1 ± 10.3 per animal. Total incidences of intestinal metaplasias detected histologically were 91% in the pyloric gland and 30% in the fundic gland (**Table 3**).

Fig. 3. Kidney tumor, HE, $\times 200$.

Fig. 5. An islet tumor positive for insulin. Immunohistochemistry, $\times 100$.

Fig. 4. Pancreatic islet tumor, HE, $\times 200$.

Fig. 6. Apocrine tumor, HE, $\times 100$.

Table 2. Incidences of Tumors after Irradiation

Effective Animal No	Gastric	Pancreas	Kidney	Apocrine	Sarcoma		Bile duct	Liver	Small intestine	Lung
					Abdominal	Skin				
82	3 (4)	15 (18)	15 (18)	9 (11)	9 (11)	5 (6)	2 (3)	1 (1)	2 (2)	1 (1)

Table 3. Data for the Incidence (%) and Number of Foci of Intestinal Metaplasia

ALP	Pylorus				Fundus			
	A	B	C	Total	A	B	C	Total
(29)	(47)	(88)	(47)	(91)	(10)	(19)	(9)	(30)
4.1±10.3	1.1±1.4	6.8±7.0	1.0±1.4	8.8±8.7	0.3±0.8	0.4±1.4	0.3±1.4	1.1±3.0

Discussion

In previous experiments we found that well differentiated adenocarcinomas of the glandular stomach developed within 1 year after one or two applications of 20 Gy of X-irradiation to the gastric region of rats⁵ and signet ring cell carcinomas in the glandular stomach were also observed after irradiation of hypocatalasemic mice with the same dose⁶. The incidence of gastric tumors was increased with two 20 Gy irradiations⁷. The results suggested that induction of gastric tumors by low dose X-irradiation requires a relatively long latent period.

In the present experiment, the observed incidence of intestinal metaplasia was decreased as compared with the previous experiment at 1 year after X-irradiation. The yield of gastric tumors was also very low. We have shown that the development and maintenance of intestinal metaplasia can be influenced by the pH value with at least partial reversibility associated with gastric alkalinity.

The high incidence of islet tumors observed in the present experiment is in agreement with previous reports. Gilbert and Gillman noted a low incidence of such tumors in 1,342 non-irradiated Wistar rats⁸, and spontaneous pancreatic islet tumors have been reported in SD rat strains⁹⁻¹⁴ but they can also be induced by X-rays or neutron irradiation within a short period¹⁵⁻²¹. These appear to be the same strain variation however. Lemson *et al.* observed pancreatic islet adenomas in 1.4% of female Wistar rats studied for late radiation effects¹⁵, while Rosen *et al.*^{16,17} reported a 9.8% incidence in control male SD rats, while those in receiving 430 and 680 rads had 19.0% and 17.2% incidences, respectively. The same tumor type was found in 5.6% of 41 animals exposed to 230 rads of fast neutrons and in 44% of rats exposed to 320 rads^{13,18}. Warren *et al.*¹⁹ rarely encountered islet cell tumors in 113 pairs of control parabiont Deac-Slonker rats (1 per cent, one male only) as non-irradiated parabionts (0.7 per cent, two out of a total of 276 males and none in the 326 females). They were more common in the male irradiated partners (12.7%) but infrequent in the female irradiated parabionts (2%). Boschetti *et al.*²⁰ reported a 11.4% incidence of islet cell tumors in non-inbred Wistar rats treated with 430 rads total body X-irradiation while the same treatment resulted in a 2.2% incidence in inbred Wistar Furth rats. In the present investigation, the tumor first appeared at 400 days after the first X-irradiation but the high yield suggests that the present protocol might be useful to establish a model for induction of insulinomas. Further studies are required to determine serum values of hormones.

The kidney tumors which appeared in this experiment are similar to those reported earlier in X-irradiated SD male rats. Thus Rosen *et al.* found 40% and 29% of rats receiving 4.3 Gy and 6.8 Gy, respectively had renal parenchymal tumors, whereas the non-irradiated controls failed to show any evidence of renal neoplasia²¹. The total incidence of renal neoplasia in Neutron-irradiation also caused an

approximately 40% yield¹⁵. The kidneys demonstrated atrophy during the 12 months after X-irradiation and the kidney tumors appeared to arise from atrophic areas suggesting a direct link with damage due to scattered X-irradiation.

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