Original Articles

INFLUENCE OF X-RAYS ON TRANSGENERATIONAL LUNG TUMORIGENESIS IN CBA MICE

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Abstract : The aim of the present study was to ascertain whether prezygotic exposure of male mice to X-rays increases a carcinogenic risk in the progeny. The testicles of 9-week-old male CBA/J mice were X-ray irradiated (1 Gy or 2 Gy) and the animals were then mated at 1, 3 and 9 weeks after treatment with untreated virgin 12-week-old females of the same strain. The 1-Gy offspring (155 males and 127 females), 2-Gy offspring (111 males and 95 females) and additional control offspring (119 males and 111 females) were kept for life under standard laboratory conditions. The lungs were investigated for development of adenomas and adenocarcinomas. No significantly increased tumour incidences were observed in the 1-Gy and 2-Gy offspring groups compared with the control group. However, with regard to the dignity of the tumours, the ratios of animals with carcinomas to animals with adenomas were slightly increased in both irradiation groups of descendants. Although this phenomenon is not statistically significant, a possibility of enhanced susceptibility to malignant progression of tumours in the offspring through the paternal germ cell X-irradiation should be considered in connection with our previous investigation.

Key words : CBA/J mice, prezygotic, testicular, X-ray irradiation, lung tumours, progeny

INTRODUCTION

In a previous investigation (Kamino and Mohr, 1998), we have shown that a prezygotic exposure of male mice to X-rays possibly induces a hypersensitivity to chemical carcinogenesis in the progeny. The aim of the present long-term study was to ascertain what may happen in the offspring of irradiated paternal parents, if they are kept for life without any further treatment. Do they preserve their own spontaneous tumour rate, or do they show an increased susceptibility to tumour development ? Regarding the possibility of germ cell alterations following exposure to ionizing radiation increasing a carcinogenic risk in the progeny, this has been discussed controversially in several epidemiological and experimental studies. In the epidemiological studies, the hypothesis of Gardner et al. (1990), i. e. that external ionizing irradiation of fathers prior to conception enhances the leukemia risk of the children, was not supported by the findings of a similar investigation by McLaughlin and co-workers (1993). Furthermore, Yoshimoto and colleagues (1990) found no increase in malignancy in the children of atomic bomb survivors. In experimental

investigations, Nomura (1982, 1991) demonstrated heritable tumours in the offspring after paternal exposure to X-ray irradiation in ICR mice. An increased frequency of liver tumours was observed in the progeny of neutron-irradiated male C3H mice before mating (Takahashi, et al., 1992 ; Watanabe et al., 1996; Shoji et al., 1998). In contrast, according to the studies of Cattanach et al. (1995, 1998), paternal X-irradiation did not affect lung tumour incidence in the offspring of BALB/cJ and C3H/HeH mice. The present paper should contribute to the active ongoing discussion.

MATERIAL AND METHODS

The testicles of 9-week-old male CBA/J mice (Charles River, Sulzfeld, Germany) were X-ray irradiated under ketamine/xylazine narcosis. An X-ray generator for skin irradiation, Philips type RT100 (Philips, Hamburg, Germany) was used, operating at 8 mA and 100 kV with a filter of 1.7 mm aluminium and 0.2 mm copper. The total dose of 1 Gy or 2 Gy was administered in two single doses of either 0.5 Gy or 1 Gy, respectively, with a 24-hour interval. One, 3 and 9 weeks after the X-ray irradiation, the parental males were mated with untreated 12-week-old virgin females of the same strain. The control paternal animals were treated with the identical narcosis but no X-irradiation and mated 1 week later in the same manner (Fig. 1). At the age of 6 weeks, all offspring were treated once subcutaneously with saline (0.01 ml/g body wt) (Mohr et al., 1999). They were housed three per cage in Makrolon Type II cages (350 cm2) and kept for their entire lifespan under standard laboratory conditions (room temperature 21 ± 2° C; relative humidity 45-70% ; air exchange 15 times/h ; 12h/12h light/dark cycle). Absorbent softwood (H3/4, Hahn & Cobedding material in the cage. The mice received a pelleted maintenance diet 1324 (Altromin GmbH & Co., Lage, Germany) and tap water ad libitum. They were checked daily and weighed weekly; moribund animals were killed with an overdose of CO_2. Uniform methods were

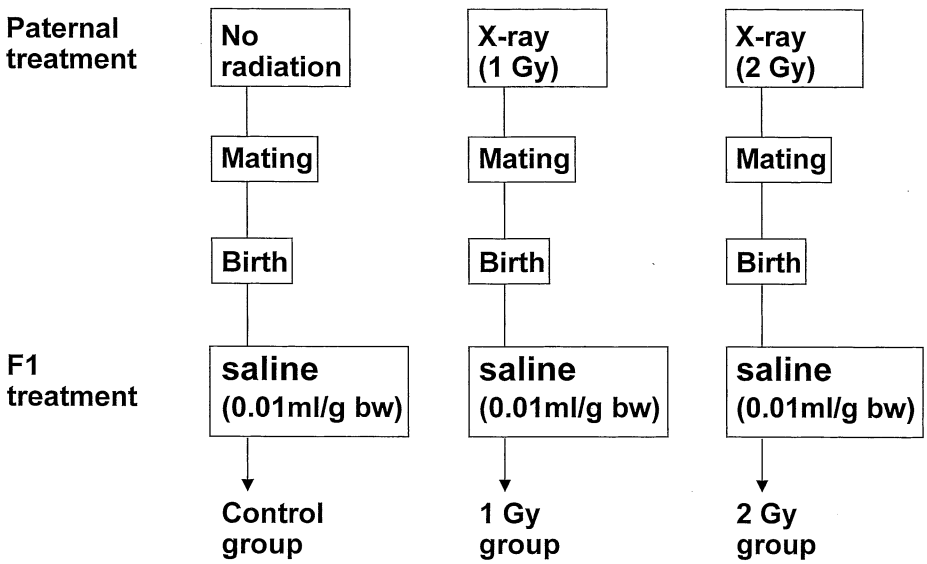

Fig. 1 Experimental design.

used for all autopsies. Lungs were examined visually and surface tumour nodules counted for each animal. After complete autopsies, all organs were fixed in 10% buffered formalin which was refreshed after 24 hours. Infusion fixation was performed on the lungs. Tissue specimens from each of five lung lobes and macroscopically visible nodules were embedded in Paraplast PlusTM (Sherwood Medical Co., St. Louis, MO, USA), sectioned longitudinally and H&E stained. Permanent checking of the lung sections for additional nodules was guaranteed during slide processing. Histological sections (3-4μm thick) of the nodules and each lobe cut at the level of the lobar bronchus were carefully examined microscopically. Even when no lesions were visible macroscopically, one slide per lung lobe was examined. In addition, numerous step sections of the lungs (200μm) were carried out, beginning at the level of the bronchi (Tillmann et al., 1999). In the histopathological diagnosis, adenocarcinomas were distinguished from the adenomas according to the common criteria for malignancy, i. e. cellular pleomorphism, enlarged and hyperchromatic nuclei with coarsely clumped chromatin and prominent nucleoli, increased and atypical mitosis, bizarre giant tumour cells, invasion and necrosis. In borderline cases, the relevant publications were referred to (Rehm et al., 1994 ; Rittinghausen et al., 1996). Tumour diagnoses were documented and evaluatedusing the "Pathology Lexicon Acquisition, Correlation and Evaluation System" (PLACES 2000), Version 1 (Apoloco Ltd., Newcastle, England). In the statistics, the two-tailed Fisher's exact test was used.

RESULTS

The number of F1 animals and the average life expectancy are shown in Table 1. Gonadal X-ray irradiation apparently decreased the number of 2-Gy offspring at the 3-week and, in particular, at the 9-week mating time-points. A significant shortness of the lifespan was observed in the female mice of the 1-Gy-9-weeks subgroup.

Table 1 Mean lifetime of the progeny (weekls)

	Control	1Gy			2Gy		
	1week	1week	3weeks	9weeks	1week	3weeks	9weeks
n	119	60	39	56	76	27	8
Male	83±19	86±13	86±116	87±112	86±19	87±18	78±17
n	111	45	38	44	69	20	6
Female	94±17	90±16	95±16	83±21**	93±17	91±17	83±19

Mean±S. D.; Student's t-test ; **p<0.01

In the lung, only bronchiolo-alveolar adenomas and adenocarcinomas occurred ; other primary lung tumours were not detected. The adenomas (Fig. 2) were well circumscribed and up to 3 mm in diameter. Not only tubular or solid but also alveolar or papillary growth patterns of tumours were observed. The proliferating epithelial cells included round or oval, relatively monomorphic nuclei with fine chromatin. Mitotic figures were mostly rare or even absent. The majority of the adenocarcinomas (Fig. 3) were irregularly circumscribed and larger than 3 mm indiameter. The tumours included numerous irregularly-shaped and in some places papillary atypical glandular formations. The glandular spaces were lined with

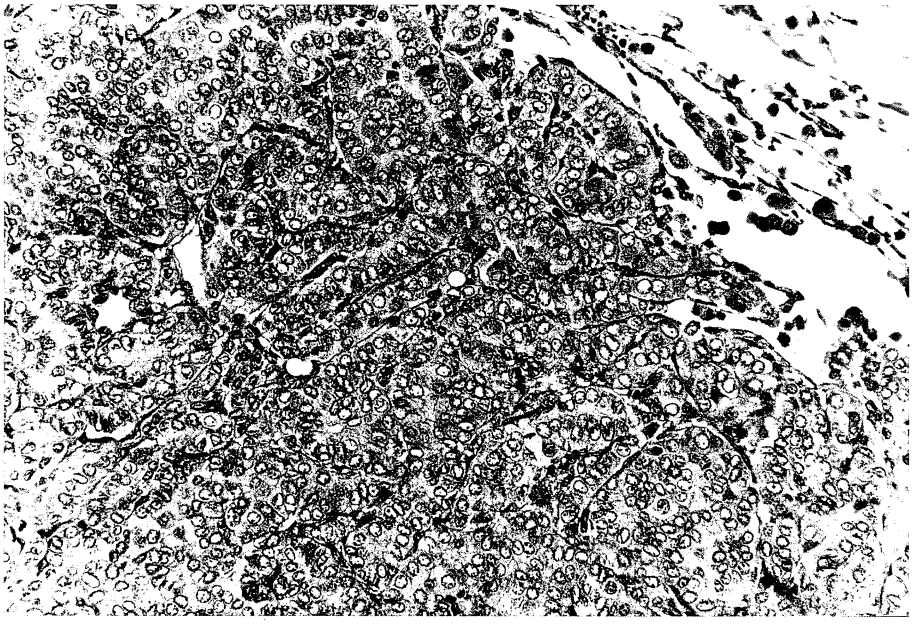

Fig. 2. Well circumscribed adenoma consisting of closely-packed glandular formations with round to oval monomorphic nuclei. H&E, x 25.

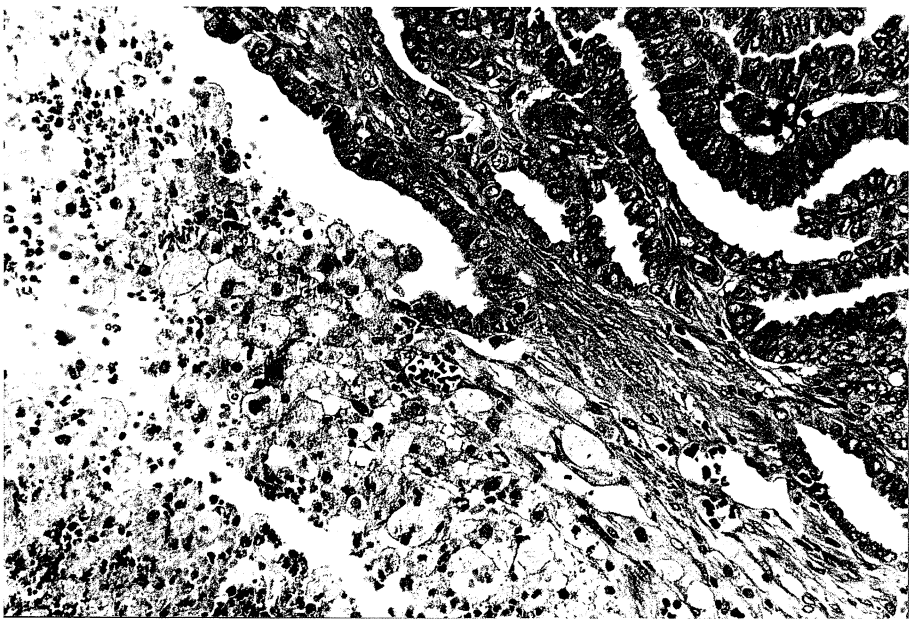

Fig. 3. Papillary area of lung adenocarcinoma with oval to elongated nuclei. Note the necrosis. H&E, x 25.

Table 2a. Incidence of lung tumours in the male progeny

	Male						
	Control	1Gy			2Gy		
		1week	3weeks	9weeks	1week	3weeks	9weeks
Total animal number	119 (100%)	60 (100%)	39 (100%)	56 (100%)	76 (100%)	27 (100%)	8 (100%)
Total tumour-bearing animals	30 (25%)	12 (20%)	7 (18%)	13 (23%)	16 (30%)	6 (22%)	2 (25%)
Animals with adenomas only	26 (22%)	10 (17%)	6 (15%)	11 (20%)	16 (21%)	5 (19%)	2 (25%)
Animals with carcinomas	4 (3%)	2 (3%)	1 (3%)	2 (4%)	7 (9%)	1 (4%)	0 (0%)
Ratio Ca / Ad	0.15	0.20	0.17	0.18	0.44	0.20	0.00

Ca : animals with carcinomas ; Ad : animals with adenomas only

Table 2b. Incidence of lung tumours in the male progeny

	Male						
	Control	1Gy			2Gy		
		1week	3weeks	9weeks	1week	3weeks	9weeks
Total animal number	111 (100%)	45 (100%)	38 (100%)	44 (100%)	69 (100%)	20 (100%)	6 (100%)
Total tumour-bearing animals	26 (23%)	4 (9%)	9 (24%)	19 (20%)	15 (22%)	5 (25%)	0 (0%)
Animals with adenomas only	25 (23%)	4 (9%)	8 (21%)	8 (18%)	12 (17%)	4 (20%)	0 (0%)
Animals with carcinomas	1 (1%)	0 (0%)	1 (3%)	1 (2%)	3 (4%)	1 (5%)	0 (0%)
Ratio Ca / Ad	0.04	0.00	0.13	0.13	0.25	0.25	0.00

Ca : animals with carcinomas ; Ad : animals with adenomas only
Fisher's exact test : $*p<0.05$;

one or two layers of cuboidal to cylindrical epithelial cells including moderately pleomorphic nuclei with slightly coarse chromatin. Nucleus/cytoplasm ratio of the atypical epithelial cells was shifted towards the nucleus. Small necrotic areas were occasionally seen within neoplastic nodules.

The incidence of the lung tumours is presented in Tables 2a and 2b. In both sexes, the tumour incidences at the 1-, 3- and 9-week mating time-points of both irradiation groups were sometimes higher, sometimes lower and showed no significant increase compared with the controls. However, with regard to the dignity of the tumours, the incidences of carcinoma were slightly higher in the 1-Gy and 2-Gy groups than in the control group ; correspondingly, the numbers of adenoma-bearing offspring were lower, and the ratios of descendants with carcinomas to mice with adenomas were slightly raised in a dose-related

manner. This phenomenon was not observed in the 1-Gy-1-week females and 2-Gy-9-week subgroups of both sexes. Furthermore, all these observations were statistically not significant.

DISCUSSION

The significant reduction in the number of litters born in the 2-Gy-3-week and, particularly, 2-Gy-9-week subgroups of both sexes indicates a dose-dependent germ cell alteration in the early stage of the spermiogenesis with regard to reproduction. It was possibly due to oligo- and pathospermia (Meistrich, 1986) as well as fetal death and resorption (Wachsmann, 1982). The statistically significant shortening of the mean lifetime in the female 1-Gy-9-week subgroup cannot be interpreted.

In the hitherto existing experimental investigations, not only with positive but also with negative results, the differences in tumour incidences between controls and offspring of exposed animals have mostly been taken into consideration (Nomura, 1982, 1991 ; Takahashi et al., 1992 ; Cattanach et al., 1995, 1998 ; Watanabe et al., 1996; Shoji et al., 1998). Looking at the present study from the viewpoint of tumour incidence, the results are clearly negative; it means that the X-ray has no effect on paternal germ cells with regard to tumour susceptibility in the progeny. Our previous study had the same results (Kamino and Mohr, 1998). The characteristic of the previous and present investigations is the special attention to the dignity of the lung tumours. Moreover, in the current study, numerous step sections from each lung lobe were made, so that, most probably, all tumours that occurred were completely examined histologically (Tillmann et al., 1999). The results were as follows: in both sexes, the ratio of carcinoma-bearing to adenoma-bearing animals was, after paternal X-irradiation, mostly higher in the offspring groups than in the control groups in a dose-related manner, even if these increases were statistically not significant. The female 1-Gy-1-week subgroup not falling in with this pattern might just be an exception, and the results in the 2-Gy-9-week subgroups of both sexes should not be taken into account because of their extremely small animal numbers.

Our previous investigation (Kamino and Mohr, 1998) also showed similar phenomena, whereby, in the male 2-Gy group, the increase of the incidence of lung carcinomas was just below statistical significance and the multiplicity of carcinomas was significantly increased. It is conceivable that, in the previous experiment, the actual observation was amplified by the additional carcinogenic treatment of the offspring with urethane. In conclusion, the present findings indicate that the paternal germ cell alterations caused by prezygotic X-irradiation seem to have no direct tumorigenic effect but a possibility to heighten the malignant degeneration of benign lung tumours in the progeny.In humans, the pointmutation of the K-ras oncogene is an important gene alteration, particularly with regard to the progression to lung adenocarcinoma (Sugio et al., 1994). However, the analysis of the lung tumours of CBA mice showed that the K-ras mutations play a role in the early stages of mouse lung tumorigenesis (Cazorla et al., 1998) but not in the progression from adenoma to carcinoma. Furthermore, the investigations of Kawano et al. (1995, 1996) using B6C3F1 and A/J mice resulted in the same conclusions as those of Cazorla and her co-workers. In these studies the K-ras mutation occurred apparently in the tumour tissue and was not available

as a germline mutation. It is known that germline mutations at hypervariable mice minisatellite loci can be induced by ionizing radiation (Sadamoto et al., 1994 ; Fan et al., 1995; Jeffreys et al., 1997). However, it is not clear whether these mutations at minisatellite loci are the cause of our observation. Further relevant investigations should be carried out to clarify what types of vertically transmitted germline alterations are responsible for this phenomenon.

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