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Co-operative effects of thoracic X-ray irradiation and N-nitrosobis(2-hydroxypropyl) amine administration on lung tumorigenesis in neonatal, juvenile and adult Wistar rats

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ABSTRACT

Assessment of risks associated with childhood exposure to ionizing radiation when combined with chemical carcinogens is of great importance. We studied the age-dependence of the effect of combined exposure to ionizing radiation (IR) and a chemical carcinogen on lung carcinogenesis. Female 1-, 5-, and 22-week-old Wistar rats were locally irradiated on the thorax with X-rays (3.18 Gy) and/or were injected intraperitoneally with N-nitrosobis(2-hydroxypropyl)amine (BHP) (1 g/kg body weight) 1 week after X-ray exposure or at 23 weeks of age. Rats were terminated at 90 weeks of age. We found that: (i) the incidence of lung tumors (adenoma and adenocarcinoma) increased slightly as a function of age at X-ray exposure, although this was not statistically significant, while the incidence induced by BHP decreased with increasing age at administration; (ii) combined exposure to X-rays at 5 or 22 weeks with BHP 1 week later enhanced the tumor incidence, and the effect at early-life stage (5 weeks irradiation) was more effective than that at late-life stage (22 weeks irradiation); (iii) combined exposure preferentially enhanced malignant transformation; (iv) although a longer interval between the X-ray and BHP treatments reduced the combined effect, risks of early-life irradiation at 1 or 5 weeks of age lasted into adulthood; (v) adenomas and adenocarcinomas induced by X-ray and/or BHP originated from surfactant apoprotein A-positive alveolar type II cells; and (vi), extracellular signal-regulated kinase pathway activation was observed in half the adenocarcinomas, regardless of the exposure schedule. In conclusion, combined exposure may enhance lung tumorigenesis more synergistically at early-life stage (5 weeks of age) than later-life stage.

Introduction

Numerous physical, chemical, and biological carcinogens are found in the environment, and quantifying the impact of combined exposure to different carcinogens is therefore a priority for ensuring human health (Cohen, 2000; Preston, 2005; UNSCEAR, 2000). One's age at the time of exposure to carcinogens is an important biological variable influencing carcinogenic effects. Several studies have reported that neonates and juveniles are more strongly affected by ionizing radiation (IR) and carcinogenic substances than adults (Gehlhaus et al., 2011; Perera, 1997; Preston, 2004).

The main cause of human lung cancer is tobacco smoking. Case–control comparisons indicate that smokers who started before the age of 15 years have double the risk of lung cancer as those who started at 20 years of age or older (Peto et al., 2000). Compared with adults, children may be more susceptible to the carcinogens in tobacco smoke because of relatively higher air intake in proportion to lung size (Armstrong et al., 2002) and less efficient carcinogen detoxification (Bearer, 1995). Available evidence on the risk of lung cancer in adulthood after childhood passive smoke exposure, however, points to no increase in risk (Boffetta et al., 2000).

A rapid increase in the use of chest irradiation from computed tomography (CT) or radiation therapy, particularly in children, is a source of growing concern among clinicians and radiation protection authorities. Because the lung is a leading site of malignancy worldwide, estimating lung cancer risk following pediatric chest irradiation

Abbreviations: BHP, N-nitrosobis(2-hydroxypropyl)amine; ERK, extracellular signal-regulated kinase.

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is crucial. Studies of atomic bomb survivors in Hiroshima and Nagasaki revealed that the risk of radiation-associated leukemia and most solid cancers is greater in the younger age exposed group than in those exposed as adults (Miller, 1984; Preston et al., 2007). However, lung cancer was an exception; excess relative risk (ERR) for lung cancer incidence in atomic bomb survivors exposed as adults is higher than in exposed juveniles (Furukawa et al., 2010; Preston et al., 2007; UNSCEAR, 2000). Preston et al. (2007) suspected that this pattern may be a consequence of the failure to adjust for the effects of smoking. However, Furukawa et al. (2010) indicated that this may not be the case. It is yet uncertain whether childhood exposure to IR and daily secondhand smoke increases the risk of lung cancer.

Because epidemiological studies cannot perfectly control for extrinsic or confounding factors, obtaining data on the age-dependence of susceptibility to tumorigenesis requires the use of a suitable animal model. We previously showed that thoracic X-ray irradiation of female Wistar rats efficiently induces lung tumors; adenomas and adenocarcinomas are predominantly induced by a low dose of 1–3 Gy in thoracic X-irradiated rats, whereas higher doses of 5–10 Gy induce adenosquamous and squamous cell carcinomas in addition to adenomas and adenocarcinomas (Oghiso and Yamada, 2000, 2003). Tobacco smoke contains many chemicals, such as nitrosamines, clearly capable of causing lung cancer. A single intraperitoneal injection of the nitrosamine, N-nitrosobis(2-hydroxypropyl)amine (BHP) efficiently induces lung adenomas and adenocarcinomas in Wistar rats (Konishi et al., 1978, 1980, 1982; Yokose et al., 1988). Thus, thoracic X-ray irradiation and BHP treatment in Wistar rats are appropriate for investigating the combined effects of radiation and nitrosamine in pulmonary tumorigenesis.

Despite the complex nature of lung cancer biology, its molecular characteristics are becoming increasingly clear. The two most commonly mutated oncogenes in human lung cancer are *KRAS* (Rodenhuis et al., 1987; Santos et al., 1984) and the epidermal growth factor receptor (*EGFR*) (Lynch et al., 2004); and, *KRAS* and *EGFR* are both members of the extracellular signal-regulated kinase (ERK) pathway. It has been shown that amplification of ERK signaling is a critical determinant of malignant progression in lung cancer (Feldser et al., 2010). *EGFR* mutations are more commonly found in tumors from patients who never smoked cigarettes (Pao et al., 2004). In animal experiments, *Egfr* mutations with amino acid substitution have been observed in exons 18 and 21 in X-ray-induced rat lung tumors (Kitahashi et al., 2008). Activating mutations of *Kras* and *β-catenin* are observed in BHP-induced tumors (Kitahashi et al., 2008; Tsujiuchi et al., 2000). Although the molecular changes in oncogenes during lung carcinogenesis are dependent on the carcinogenic agent, little is known about which mutations are involved in lung carcinogenesis induced by combined radiation and chemical carcinogens.

In this study, we examined the combined effects of thoracic X-ray exposure and a single intraperitoneal injection of BHP on pulmonary tumorigenesis in neonatal, juvenile, and adult rats with a short or long interval between treatments. We also investigated whether *Egfr*, *Kras* and *β-catenin* mutations, as well as activation of ERK, are detected in X-ray-BHP co-induced cancers.

Materials and methods

Animals. Female specific-pathogen-free Wistar rats (WM/Nrs) were purchased from a breeding facility (Japan SLC Co.) when pregnant, or 4 or 6 weeks after birth, and they or their litters were irradiated at the age of 1, 5, or 22 weeks. The animals were housed four per aluminum cage with wood chip bedding and were supplied with a commercial diet (CE-2; CLEA Japan Inc.) and disinfected water (chloride concentration 10.0 ± 2.0 ppm; pH 3.0 ± 0.2) ad libitum. All animal rooms were maintained with barrier-filtered air on a 12-h light:dark cycle at a temperature of 23.0 ± 2.0°C with 50 ± 10% humidity. This study was performed with the approval of the institutional animal care and use committee of the National Institute of Radiological Sciences.

X-ray irradiation and dosimetry. Thoracic X-ray irradiation was performed using a modified version of a published procedure (Oghiso and Yamada, 2003). The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (30–33 mg/kg body weight), and control (0 Gy) animals were also anesthetized for sham exposure. The 5- or 22-week-old rats were placed in an acrylic holder in a prone position. The holders were shielded with a 6-mm-thick lead sheet containing a 30- or 40-mm-wide slit to define the exposure area of the thoracic region of the 5- and 22-week-old rats, respectively. The 1-week-old rats were placed on an acrylic plate in a prone position, and each animal was shielded with a 6-mm-thick lead sheet containing a window (20 × 10 mm) to define the exposure area of the thoracic region.

The animals were irradiated with an X-ray unit (PANTAK HF-320, Shimadzu, Japan) operated at 200 kVp and 20 mA, with 0.5 mm copper and 0.5 mm aluminum filters, and a filament-substance distance of 750 mm. The exposure dose rate was 0.540–0.545 Gy/min, as measured with a dose-rate meter (AE-1321M, Ohyo Giken Co. Ltd., Japan) coupled to an ionization chamber (C-110, 0.6-ml volume, Ohyo Giken Co. Ltd.).

Chemical compound and treatments. BHP (CAS No. 53609-64-6, Nacalai Tesque, Inc., Japan) was dissolved in saline at a final concentration of 200 mg/ml and administered as a single intraperitoneal injection at a dose of 1 g/kg body weight in a safety cabinet with forced ventilation. Vehicle controls were injected with saline alone. BHP-injected animals were placed in disposable cages and kept in the safety cabinet for 24 h for protection against chemical contamination. After 24 h, the animals were transferred into new disposable cages and kept in separate cage racks for one month. The animals were then placed in normal aluminum cages in ordinary cage racks.

A total of 248 rats were divided into twelve experimental groups: untreated control; irradiated with X-rays (3.18 Gy) at 1, 5 or 22 weeks old (groups A, B, and C, respectively); injected with BHP (1 g/kg body weight) at 2, 6 or 23 weeks old (groups D, E, and F, respectively); irradiated with X-rays at 1 week old and injected with BHP at 2 weeks old (group G); irradiated with X-rays at 1 week old

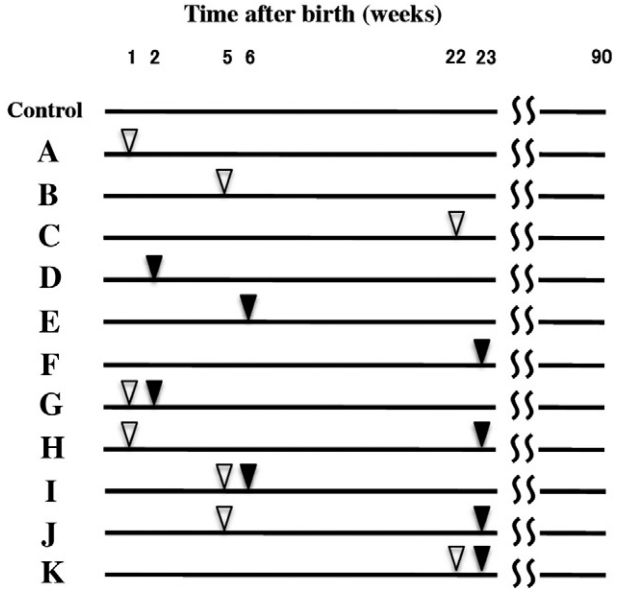

Fig. 1. Experimental design. Open triangles indicate thoracic X-ray irradiation (3.18 Gy). Closed triangles indicate BHP administration (i.p., 1 g/kg body weight). Animals were terminated at 90 weeks.