Compensatory Lung Growth after Partial Pneumonectomy Enhances Lung Tumorigenesis Induced by 3-Methylcholanthrene

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Abstract

In small mammals, partial pneumonectomy (PNX) elicits rapid hyperplastic compensatory growth of the remaining lung parenchyma to restore normal lung mass, structure, and function. In BALB mice subjected to PNX, compensatory lung growth is complete within 10 days. Because cellular hyperplasia contributes to the mechanism of tumor promotion by butylated hydroxytoluene (BHT), we hypothesized that hyperplastic compensatory lung growth would promote tumor formation in carcinogen-treated animals in a manner similar to that observed after BHT. In mice subjected to PNX, within 1 week of treatment with the carcinogen 3-methylcholanthrene (MCA; 10 μg/g body weight), lung tumor multiplicity was 3–7-fold higher in animals subjected to PNX than in mice subjected to a sham operation. The increase in tumor multiplicity occurred when PNX was performed 1, 3, and 6 days before or 1 day after MCA treatment. In the absence of PNX, lung tumor multiplicity in MCA-treated mice given one injection of BHT (200 mg/kg body weight) increased significantly \( (P < 0.01) \) as compared to that in mice given MCA alone. Tumor multiplicity continued to increase linearly \( (R^2 = 0.99) \) with each subsequent BHT injection. Lung tumor multiplicity and tumor size in mice given one or two injections of BHT were comparable to those in animals subjected to PNX. These data demonstrate that post-PNX compensatory lung growth stimulates tumorigenesis in MCA-treated mice and provides a novel model for investigating tumor formation.

Introduction

PNX\(^1\) in small mammals initiates a rapid and diffuse compensatory hyperplasia in the remaining lung parenchyma to restore normal total mass, structure, and function \( (1) \). The characteristics of compensatory lung growth are similar among different mammalian species; although gender, age, and hormonal status modulate the growth response \( (2) \). Upon restoration of total lung mass, alveolar number and function appear normal, and both alveolar and capillary surface areas are comparable to controls \( (1) \). Events that initiate the growth response have been subject to much investigation; elevated blood flow to the remaining lobes, increased inflation of the remaining lobes, and release of soluble growth factors have been considered, but no single event has been identified as causal \( (1, 2) \). A recent report suggests that mechanical signals, such as stretch of the remaining right lung, may be important in the initiation of the growth response. Expression of some immediate early genes is significantly higher in the remaining lung 30 min after PNX, and this effect is mimicked in lungs subjected to increased inflation \( (3) \).

Because the promotion phase of carcinogenesis involves cellular proliferation and clonal expansion of initiated cells, we reasoned that the hyperplastic growth response after PNX could also promote lung tumor formation in carcinogen-treated mice and perhaps mimic chemical tumor promoters that cause lung injury. One such promoter, BHT, is an antioxidant used commercially as a food additive. In mice, BHT exposure elicits acute lung injury characterized by alveolar type I epithelial cell death, pulmonary edema, and macrophage infiltration \( (4, 5) \). Subsequently, compensatory hyperplastic cell growth ensues in which alveolar type II epithelial cells divide and differentiate into type I cells \( (4) \). Within 1 week after BHT exposure, the injury is repaired, and lung histology is normal \( (5) \).

Although BHT itself is not genotoxic or carcinogenic \( (6) \), it can modulate tumor formation. In mice, BHT can prevent or enhance lung tumor formation, depending on the inbred strain and age and on whether BHT administration precedes or follows carcinogen exposure \( (7–9) \). Tumor promotion by BHT in rodents is achieved by repeated weekly administration of the agent after a single carcinogen exposure \( (8–10) \).

The effect of PNX on pulmonary tumorigenesis was investigated by administering the carcinogen MCA either during or before compensatory lung growth. Lung tumor multiplicity in PNX mice was compared to that in mice treated with the tumor promoter BHT. The results of these experiments demonstrate that post-PNX compensatory lung growth increases lung tumor multiplicity in carcinogen-treated mice in a manner similar to acute BHT treatment.

Materials and Methods

Animals. Male BALB/cByJ (BALB) mice obtained from The Jackson Laboratory (Bar Harbor, ME) at 5–6 weeks of age were housed 3–5 mice/cage and kept in the pathogen-free Animal Resource Facilities at Hershey Medical Center. Animals were maintained on corn cob bedding and fed Harland Teklad Rodent Diet and water ad libitum.

Pneumonectomy Procedure. Mice were weighed and anesthetized with ketamine (125 mg/kg body weight) and xylazine (10 mg/kg body weight) in 0.9% NaCl solution at a dosage of 0.1 ml/20 g body weight. Animals were restrained on an inclined surgical board, and hair was trimmed away from the left side of the chest. A cutaneous incision was made from the xyphoid process to the left axilla, followed by a second incision in the intercostal space between the fourth and fifth ribs. Using a forceps, the left lung was gently lifted through the opening, the main bronchus was tied with surgical silk, and the lung was removed by cutting distal to the ligature. This procedure removes approximately 35% of total lung mass. Immediately after left PNX, the chest cavity was closed with sutures, and the surgery-induced pneumothorax was reduced by inserting a hypodermic needle through the chest wall and withdrawing air until negative pressure was achieved. The skin was closed with staples, and mice were allowed to recover on a warm thermal pack before being returned to their cages. SHAM animals underwent a thorocotomy, but the lung was not ligated or removed. Mice were sacrificed by lethal injection of sodium pentobarbital (60 mg/kg body weight) at specific intervals after surgery, and lungs were removed from mice and weighed.

Tumor Promotion Protocol. All mice were given a single i.p. injection of MCA (10 μg/g body weight; Sigma) and subsequent i.p. injections of BHT (200 mg/kg body weight; Sigma) once a week for 1, 2, 3, or 6 weeks beginning...
7 days after the initial MCA injection. Control groups were treated with MCA alone or with MCA followed by i.p. injections of corn oil vehicle (100 μl).

For experiments investigating compensatory lung growth as a tumor promoter, mice were subjected to PNX at various intervals after exposure to the carcinogen MCA. Additionally, mice were subjected to PNX at various intervals before MCA exposure. Thus, in the latter experiment, carcinogen exposure occurred at early, middle, and late stages of compensatory lung growth. In both cases, SHAM mice served as controls.

**Lung Fixation.** Mice were sacrificed 16 weeks after MCA exposure by lethal i.p. injection of sodium pentobarbital (60 mg/kg body weight). Lungs were inflated with 10% neutral buffered formalin using a constant hydrostatic pressure of 25 cm H2O for 1 min. Lungs were subsequently removed from the chest cavity and submerged in formalin overnight. Fixed lungs were transferred to 95% ethanol, the lobes were separated, and non-parenchymal tissue was removed and discarded.

**Quantification and Measurement of Tumors.** Samples were assigned a random number before counting to prevent bias. Tumors appearing as round, ivory-colored protrusions on the lung surface were counted using a dissecting microscope, and the diameter of each was measured using a 10-mm eyepiece micrometer with 0.1 mm increments. After tumor quantitation, lungs were embedded in paraffin, sectioned, and stained with H&E. Sections were classified as either solid or papillary adenomas using standard criteria (11, 12).

**Statistical Analysis.** Lung tumor multiplicity was determined by dividing the sum of the lung tumors by the number of animals in each group. Tumor incidence is defined as the percentage of mice per group with at least one tumor. Statistical significance was determined using a two-tailed Student’s t-test; values were considered to differ significantly when P < 0.05. Data represent the mean ± SE.

**Results**

Lung tumor multiplicity was 0.5 ± 0.2 in mice after a single dose of MCA (Fig. 1) and in MCA-treated mice given six weekly injections of the corn oil vehicle (data not shown). With the addition of 1, 2, 3, or 6 weekly BHT injections, multiplicity increased 6-fold (3.0 ± 0.6), 10-fold (4.9 ± 1.5), 15-fold (7.4 ± 1.5), and 27-fold (13.7 ± 1.9), respectively, as compared with mice given MCA alone (P < 0.02). Furthermore, tumor incidence in BHT-treated mice was 100% as compared to 37% in MCA-treated mice that were not given BHT. The linearity of this response (R2 = 0.99) demonstrates that BHT has a dose-frequency-dependent effect on lung tumor multiplicity in MCA-treated mice. Most lung tumors examined histologically were solid adenomas; the remainder were of a mixed or papillary morphology and were predominately found in BHT-treated mice.

The time course of accumulation of right lung weight after left PNX in BALB mice was determined (Fig. 2). Mice were subjected to either PNX or sham operations and sacrificed 1, 3, 5, 7, 10, or 14 days after surgery. Both SHAM total lung weight and right lung weight remained stable over the 14-day study. However, right lung weight from PNX mice increased markedly 1 day after surgery and continued to increase steadily until postsurgical day 10, when PNX right lung weight was not statistically different from the SHAM total lung weight. These data demonstrate rapid and complete compensatory lung growth in BALB mice.

To compare the effect of compensatory lung growth on tumor multiplicity in MCA-treated mice with that of the chemical tumor promoter BHT, animals were subjected to either PNX or sham operations before or after exposure to the carcinogen MCA. Animals that were not operated on had a lung tumor multiplicity of 0.5 ± 0.2 (Fig. 3, Un-op). When PNX was performed 1 day after exposure to MCA, lung tumor multiplicity was 2.4 ± 0.6, a 3-fold increase as compared with the value for the corresponding SHAM group (Fig. 3A). The average number of tumors in PNX and SHAM mice when surgery was performed 3 or 6 days after MCA exposure was not statistically different. Tumor incidence in PNX groups ranged from 70–93%, compared with 33–64% in SHAM mice.

Conversely, when PNX occurred 1, 3, or 6 days before MCA exposure, lung tumor multiplicity was 4.3 ± 1.1, 3.9 ± 1.0, and 6.1 ± 1.7, respectively, equivalent to a 7-fold increase over the corresponding SHAM controls (Fig. 3B). Tumor incidence was 75–88% in PNX mice and 36–46% in the SHAM mice. In contrast, neither lung tumor multiplicity nor incidence was significantly different from the SHAM values when PNX was performed 14 days before the injection of MCA. Taken together, these data suggest that compensatory lung growth can act as a tumor-promoting stimulus when PNX is performed after MCA treatment or in a manner similar to a cocarcinogen when PNX is performed before MCA treatment.

To compare effects of BHT and PNX on tumor size, lung tumor diameters were measured (Table 1). Tumor diameter was 0.21 ± 0.04 mm in mice treated with MCA alone; similar values were observed in mice subjected to a sham operation either before or after treatment with MCA. However, mice treated with two BHT injections had larger tumors (0.32 ± 0.02) than animals treated with MCA alone.
Thus, the mouse is a clinically relevant model for the study of lung cancer.

Organ growth has previously been shown to stimulate tumorigenesis in carcinogen-treated animals. For example, both the initiation and promotion phases of hepatocarcinogenesis can be influenced by liver regeneration after partial heptectomy. Male BALB/c mice subjected to partial heptectomy followed by treatment with the carcinogen urethane (ethyl carbamate) increased their heptoma incidence to 41% as compared with 0% in mice given urethane alone (16). In the present study, mice treated with MCA during the compensatory lung growth response had markedly more lung tumors than mice treated before PNX (Fig. 3). Partial heptectomy has been shown to modulate tumor formation many weeks after surgery. Rats subjected to partial heptectomy 8–10 weeks before N-nitrosodimethylamine exposure had a shorter latency period for tumor formation than similarly treated nonhepatectomized animals (17). In contrast, no significant increase in either tumor incidence or multiplicity was observed in mice subjected to PNX 2 weeks before injection with MCA (Fig. 3B).

The dose-frequency-dependent effect of BHT injections on lung tumor multiplicity has not been demonstrated previously. In A/J mice, six weekly BHT injections after a single dose of urethane markedly increased lung tumor multiplicity (18). Lung tumor multiplicity rose slightly in SWR mice given one or two weekly BHT injections as compared with mice injected with the corn oil vehicle. These differences in tumor multiplicity became statistically significant only when mice were treated with at least four BHT injections (8), suggesting that chronic exposure to BHT was necessary to enhance lung tumor multiplicity. In the present study, however, one BHT injection significantly increased tumor multiplicity after a single low dose of MCA in BALB mice (Fig. 1), suggesting that the nature of BHT effects on lung tumorigenesis can be both carcinogen and strain dependent. Tumor multiplicity in the MCA/6 BHT group was more than 2-fold higher (13.7 ± 1.9 versus 5.3 ± 1.4 tumors/mouse) than that reported previously (19), possibly because the present study used male instead of female animals. Susceptibility to urethane-induced tumor formation can be affected by gender, with male mice having more tumors than females (20).

Both BHT-treated and PNX mice developed tumors that were larger than those in animals given MCA alone or those subjected to a sham operation. Larger tumors were observed in neonatal BALB/c mice exposed to urethane than in similarly treated adult mice (11). This size difference may be attributable to factors associated with rapid developmental lung growth in neonates. Lung tumor multiplicity did not increase in neonatal mice in response to BHT treatment but did increase in adult mice (11). Members of the cytochrome P450 family

Fig. 3. Compensatory lung growth increases lung tumor multiplicity in MCA-treated mice. Mice were given a single i.p. injection of MCA (10 μg/g body weight) and subjected to PNX or sham operations 1, 3, or 6 days later (A), or mice were subjected to PNX or sham operations 14, 6, 3, or 1 day before a single injection of MCA (B). As a control, some mice were given a single MCA injection but were not subjected to surgery (Un-op). Animals were sacrificed 16 weeks after MCA injection, and lung tumors were enumerated. Data represent the mean ± SE from two separate experiments in which n = 10–17 in the PNX groups and n = 11–15 in the SHAM groups.

(P < 0.05). The largest tumor diameters were observed in mice treated with six BHT injections (0.38 ± 0.01) and were nearly twice the size of tumors observed in the animals given MCA only. In comparison, mice subjected to PNX either before or after treatment with MCA had mean tumor diameters of 0.35 ± 0.01 and 0.31 ± 0.01, respectively; both values were statistically larger than those in the corresponding SHAM group (P < 0.02) or in mice treated with MCA alone (P < 0.05). Lung tumor diameter in PNX mice was similar to that in mice given two BHT injections.

Discussion

This is the first report of post-PNX compensatory lung growth stimulating lung tumorigenesis in carcinogen-treated animals. Both lung tumor multiplicity and tumor diameter in mice subjected to PNX were comparable to mice given one or two injections of the chemical tumor promoter BHT. Lung tumors that arise within a few months after carcinogen exposure and subsequent BHT treatment are adenomas with either a papillary or a solid morphology (11, 12). Papillary and solid adenomas are thought to arise from Clara cells or type II epithelial cells, respectively (13). Human lung adenocarcinomas are also thought to arise from Clara cells and type II cells (14), and like mice, a majority these tumors harbor mutations in the k-ras gene (15).
of enzymes are responsible for the metabolism of BHT to active forms (21); therefore, this lack of enhanced tumor multiplicity in neonatal mice may be due to reduced expression of cytochrome P450 enzymes in the younger animals (22).

In conclusion, post-PNX compensatory lung growth in mice is a unique and novel model for studying lung tumor formation in carcinogen-treated animals. Although compensatory lung growth has been best studied in the rat, the mouse may prove to be a more powerful model because of the commercial availability of inbred and genetically altered mice. Compensatory lung growth has been reported in BALB/c mice (23) and observed in C57BL/6, A/J and CXB mice (data not shown). BALB mice were chosen for the present studies because of their great sensitivity to modulation of carcinogenesis by nongenotoxic agents (9, 11, 18, 19). Rapid lung growth after PNX may be a valuable substitute for traditional pneumotoxic tumor promoters, such as BHT, which result in lung injury after treatment. Post-PNX lung growth provides a mechanism to promote lung tumor formation without the complication of lung injury. Thus, compensatory lung growth will prove to be an important tool in further elucidating the mechanisms of initiation and promotion of lung cancer.

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References

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