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Induction of Squamous Metaplasia, Dysplasia and Carcinoma *in situ* of the Mouse Tracheal Mucosa by Inhalation of Sodium Chloride Mist Following Subcutaneous Injection of 4-Nitroquinoline 1-Oxide

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Squamous metaplasia, dysplasia and carcinoma *in situ* (CIS) were induced in the ICR/Jcl mouse tracheal mucosa by exposure to a mist of 5% NaCl solution following single subcutaneous injection of 4-nitroquinoline 1-oxide (4-NQO). Either subcutaneous injection of 4-NQO or NaCl inhalation alone did not cause any marked change in the tracheal mucosa. The NaCl inhalation is considered to have promotion-like action on the mouse tracheal mucosa after administration of 4-NQO injection.

Key words: Promoter — Sodium chloride — 4-Nitroquinoline 1-oxide — Tracheal Mucosa — Carcinoma *in situ*

The promoting effects of NaCl on gastric carcinogenesis have been experimentally confirmed.[1,2] High lung cancer mortality was observed in areas along the seacoast in Japan and along the northeast coast of Florida.[3,4] Excess risk of lung cancer was also observed in fishermen.[4] The exact reason for such clustering is not known, but these descriptive epidemiologic data suggest an association of lung cancer with the inhalation of NaCl. In order to examine this hypothesis, we studied the promoting effect of NaCl solution on epithelial cells in the respiratory system in mice following a single subcutaneous injection of 4-NQO.

ICR/Jcl mice (6-week-old) were divided into three groups. Groups of 28 male and 28 female mice were made to inhale an NaCl solution mist following a single subcutaneous injection of 4-NQO in olive oil at a dose of 15 mg/kg (group A). The same number of control mice received 4-NQO alone at a dose of 15 mg/kg subcutaneously (group B). Eighteen male and 18 female mice were injected subcutaneously with olive oil alone, and made to inhale the NaCl solution mist under the same conditions as group A (group C). Inhalation was performed using an ultrasonic nebulizer (OMRON NE-U11, Tateishi Electric Co., Tokyo), with a flow rate of 15 liter/min. A total of 100 ml of 5% NaCl solution was used for exposure in the inhalation chamber (400×800×300 mm, 96 liter) for 2 h per day. The size distribution of NaCl mist droplets in this chamber was measured using the Andersen Air Sampler (Dylec, Tokyo), and the mode of mist droplet size was 1.1 to 1.8 μm. Mice were exposed 5 days every week for 6 months. All mice were autopsied at 6 months from the beginning of the experiment, and 19 mice that died during the experiment were excluded from this study. Causes of death of these mice were 4 pneumonia, 4 leukemia, 1 ileus, 1 sarcoma of heart, and others unknown. At the end of the experiment, all the mice were killed, the whole trachea and lungs were fixed by 10% formalin infusion, and a longitudinal section from larynx to main bronchi was observed. All nodules in the lung were counted under a dissecting microscope and histologically confirmed. Statistical analysis was done by employing Fisher's exact test and Student's t test.

Tracheal mucosa of groups B and C retained normal ciliated epithelial cells (Fig. 1a), while those in group A showed various changes, such as squamous metaplasia (Fig. 1b), dysplasia (Fig. 1c) and CIS (Fig. 1d and Fig. 2). Keratinization and papillomatous growth were also present only in group A. Mild thickening of mucosa mainly due to so-called reserve cell hyperplasia with intermingled inflammatory cells was not regarded as squamous metaplasia, because these changes were considered to be induced by inflammation.

In the mice of group A, the tracheal mucosa had changed in 9 of 22 male mice (40.9%) and 3 of 25 female mice (12%), while only mild metaplasia was present in two mice in group B, and no remarkable changes were recognized in the mice of group C (Table I). A statistically significant difference in the occurrence of mucosal changes between group A and other groups was present in male mice ($P<0.01$). Male mice had a significantly higher incidence of mucosal changes compared to that of female mice ($P<0.05$). Dysplasia was

Abbreviations: 4-NQO, 4-nitroquinoline 1-oxide; CIS, carcinoma *in situ*.

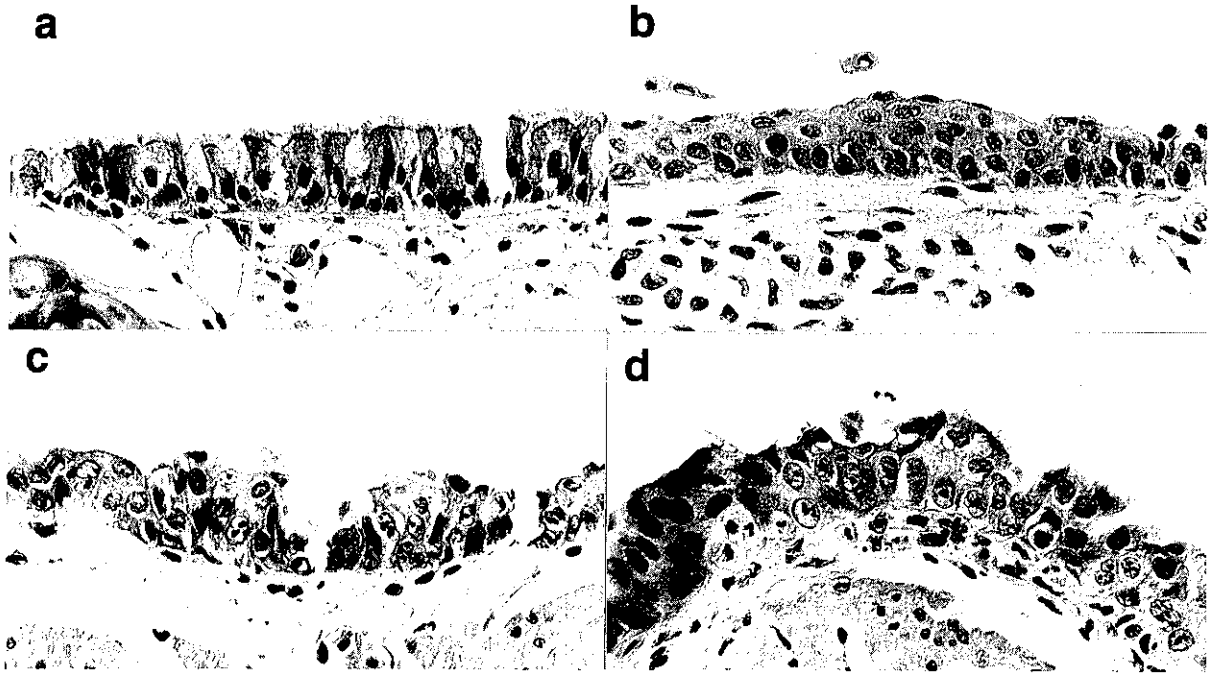

Fig. 1. Changes of tracheal mucosa of mice. (a) Non-affected mucosa in mice injected with 4-NQO. (b) Squamous metaplasia; the mucosa is thick and covered with flattened surface cells. (c) Severe dysplasia; irregular nuclei, prominent nucleoli and increased nuclear/cytoplasmic ratio are apparent. (d) CIS; epithelial cells with large nuclei and prominent nucleoli proliferate in the mucosa. Mitotic figures are frequent. Illustrations b, c and d show the trachea of male mice treated with 4-NQO injection and NaCl inhalation. (H-E, ×470).

only present in group A, and the frequencies were 36.4% in male mice, and 8% in female mice.

Induction of squamous metaplasia and tumors in the tracheal mucosa was reported in hamster, rat, dog, and others,[5-8] but it was very rare in mice. Mucosal changes were induced by exposure to chemical carcinogens,[5, 6] prolonged cuffed intubation,[7] or vitamin A deficiency.[8] In the present study, the prolonged exposure to a mist of NaCl solution induced marked changes in the tracheal mucosa only in the mice given a single injection of 4-NQO. We could not examine the promoting effect of water mist, if present. An injection of 4-NQO induced neoplasms in the lung of rats and mice.[9, 10] On this occasion, stimulated proliferation occurred in the epithelial cells lining the alveolar ducts and terminal bronchioles. Tracheal epithelial cells should have been initiated as well as bronchiolar cells, because NaCl inhalation stimulated proliferation of tracheal cells in the present report. The reason for the difference of tracheal changes by sex was not clarified, but such a difference of frequency between males and females may be attributable to hormonal and/or metabolic state. More extensive squamous metaplasia was predominantly recognized in vitamin A-deficient male rats.[11] Bronchial changes were not conspicuous as far as we examined. We consider the direct effect of mist particles may be less at bronchial mucosa, because the mode of particle size in the chamber was 1.1 to 1.8 μm and this size should increase in the

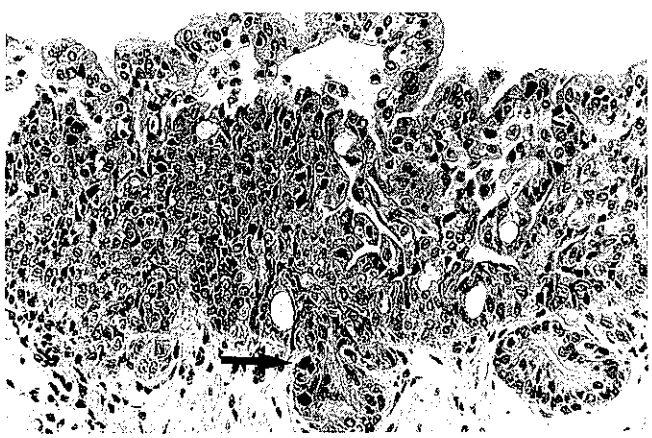

Fig. 2. Tracheal mucosa of mouse treated with 4-NQO and NaCl mist; dense proliferation with atypical mitosis (arrow) is apparent. (H-E, ×240).

Table I. Frequency of Abnormal Changes in the Tracheal Mucosa and Tumors in the Lung

	Groups (treatment)					
	A (4-NQO + NaCl)		B (4-NQO only)		C (NaCl only)	
	M	F	M	F	M	F
Trachea						
Number of mice examined	22	25	26	27	15	14
Incidence of changes[a]	9	3	1	1	0	0
(%)	(40.9)[b, c]	(12.0)	(3.8)	(3.7)	(0)	(0)
Incidence of dysplasia	6	2	0	0	0	0
(%)	(27.3)	(8.0)	(0)	(0)	(0)	(0)
Incidence of CIS	2	0	0	0	0	0
(%)	(9.1)	(0)	(0)	(0)	(0)	(0)
Lung						
Number of mice with tumor (%)	3[d] (13.6)[e, f]	12 (48.0)	16 (61.5)	13 (48.1)	2 (13.3)	0 (0)
Mean number of tumors per mouse	0.14[g]	0.68	1.38	1.04	0.13	0

a) Including metaplasia, dysplasia and CIS.
b) Significantly high incidence compared to that of female mice in group A ($P<0.05$, Fisher's exact test).
c) Significantly high incidence compared to that of male mice in groups B and C ($P<0.01$, Fisher's exact test).
d) One adenocarcinoma was included.
e) Significantly low incidence compared to that of female mice in group A ($P<0.05$, Fisher's exact test).
f) Significantly low incidence compared to that of male mice in group B ($P<0.01$, Fisher's exact test).
g) Significantly low incidence compared to that of female mice in group A or B, and that of male mice in group B ($P<0.01$, Student's t test).

airway.[12] The occurrence of lung tumors was clearly reduced NaCl inhalation (Table I). The reason for the decreased frequency of lung tumors in male mice given 4-NQO plus NaCl is unknown. Immunological enhancement, changes of microenvironment, changes in metabolism, and other factors may be involved. Most of the decrease in tumors in the lung was in adenoma, while apparent adenocarcinoma was found only in group A males. Further experiments are in progress.

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