Short Communication

Incidence of Respiratory Metaplasia of the Nasal Gland Epithelium in Untreated F344 Rats and BDF1 Mice Subjected to Two-Year Carcinogenicity Studies

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Abstract: The incidence, distribution, and histopathology of respiratory metaplasia of the nasal gland epithelium were examined in F344 rats and BDF1 mice used as untreated control animals in 2-year carcinogenicity studies. Lesions were detected in almost all the male and female rats, while they were only detected in 67.8% and 46.6% of the male and female mice, respectively. In rats, most lesions were located in the septal and/or nasoturbinate subepithelial glands at the incisive papilla level, whereas the lesions varied in location and were histopathologically more severe in mice. (J Toxicol Pathol 1998; 11: 209~212)

Key words: respiratory metaplasia, nasal gland, Bowman's gland, nasal mucosa, F344 rat, BDF1 mouse

The nasal passage is an important target site in inhalation toxicology studies. In our laboratory, attention has been paid to histopathology of the nasal mucosa in all toxicology studies including carcinogenic inhalation studies¹⁻³, since various inhaled toxicants have been reported to induce neoplastic or nonneoplastic nasal lesions in rodents⁴⁻¹¹, and various lesions were produced depend on aging^{12,13}. Although there is some information on spontaneous nasal neoplasms in rodents¹²⁻¹⁵, there are very few reports concerned with nonneoplastic lesions^{2,3}. In our previous paper^{1,2}, spontaneous lesions in Fischer 344(F344) rats and BDF1 mice were simply reported, also respiratory metaplasia of the nasal glands were described in both Bowman's glands and the nasal glands of the respiratory epithelial regions. Although they have not been described in the diagnostic criteria^{16,17}.

This paper describes the incidence, distribution, and histopathology of respiratory metaplasia in the nasal gland epithelium of untreated F344 rats and BDF1 mice observed in 2-year carcinogenicity studies.

A total of 500 male and 500 female F344/DuCrj rats and Crj:BDF1 (C57BL/6NCrj×DBA/2NCrj) mice from the non-treated groups in ten 104-week carcinogenicity studies conducted over 10 years (1986 through 1995) were used. Four-week-old male and female rats and mice were purchased from Charles River Japan, Atsugi, and following quarantine and acclimatization for 2 weeks, they were individually housed in suspended stainless-steel wire-mesh cages.

Animals were cared for according to the principles outlined in the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health, Bethesda, U.S.A. Room temperature and humidity were maintained at 23±2°C and 55±10%, respectively, with a 12-hr (08:00-20:00)-light/12-hr (20:00-08:00)-dark cycle. Tap water and commercial pellets or powder (CRF-1, γ -irradiated with 30 KGy, Oriental Yeast, Tokyo) were given *ad libitum*.

Autopsy was performed on all animals found dead or killed in a moribund condition as well as those sacrificed according to schedule. The frontal bones including the nasal tract were fixed in 10% neutral-buffered formalin for 1 to 12 weeks and decalcified in a 5% formic acid-formalin solution for 7 days. The samples were then divided into three parts at the following three cut levels as previously described¹: level I, posterior edge of the upper incisor teeth; level II, incisive papilla; and level III, anterior edge of the upper molar teeth. Paraffin-embedded sections 4 to 5 μ m thick were cut and stained with hematoxylin and eosin for microscopic examination. The incidence of the lesions was calculated for the total number of animals in the non-treated control groups including those sacrificed on schedule as well as those found dead or sacrificed in a moribund condition. The dorsal wall of the nasal cavity, the nasal septum, and the nasoturbinate or ethmoturbinate in levels I to III of the nose were examined for distribution of the lesions.

The incidences of respiratory metaplasia of the nasal subepithelial gland epithelium is shown in **Table 1**. In rats, 467 (93.4%) of the 500 males and 480 (96.0%) of the 500 females were found to have the lesions without significant variation in incidence among the studies (80.0-98.0% in males and 90.0-100% in females). In mice, the incidences were 339 (67.8%) and 233 (46.6%) of the 500 males and females, respectively, with great variability among the studies (50.0 to 86.0% in males and 21.0 to 68.0% in females).

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Table 1. Incidence of Respiratory Metaplasia of the Nasal Gland Epithelium

Animal	Male		Female	
	No. cases/No. examined	% (range) ^{a)}	No. cases/No. examined	% (range)
F344 Rat	467/500	93.4 (80.0–98.0)	480/500	96.0 (90.0–100)
BDF1 Mouse	339/500	67.8 (50.0–86.0)	233/500	46.6 (21.0–68.0)

^{a)} range from 10 studies**Table 2.** Localization of Respiratory Metaplasia of the Nasal Gland Epithelium

Site		F344 Rat		BDF1 Mouse	
		Male	Female	Male	Female
Level I ^{a)}	Dorsal wall (RE ^{d)}	3.2 (0–14.3) ^{e)}	6.5 (0–14.0)	33.3 (3.9–65.1)	24.9 (0–55.6)
	Nasal septum (RE)	0.9 (0–5.0)	2.1 (0–10.0)	38.1 (0–81.4)	37.3 (0–81.5)
	Nasoturbinate (RE)	0.6 (0–4.1)	0.8 (0–4.3)	30.4 (0–67.4)	15.0 (0–41.7)
Level II ^{b)}	Dorsal wall (OE)	0.4 (0–2.2)	1.0 (0–8.5)	16.2 (2.6–27.9)	0.9 (0–5.6)
	Nasal septum (RE)	93.0 (83.0–100)	90.4 (73.9–98.0)	44.5 (21.4–62.8)	36.9 (22.2–32.7)
	Nasoturbinate (RE)	85.7 (77.5–93.8)	73.3 (63.0–84.0)	50.0 (12.0–61.5)	30.5 (3.7–85.8)
Level III ^{c)}	Dorsal wall (OE)	0.2 (0–2.1)	0	37.2 (17.9–51.2)	6.0 (0–17.6)
	Nasal septum (OE)	0.2 (0–2.1)	0.8 (0–8.3)	15.0 (3.6–34.6)	1.3 (0–2.9)
	Ethmoturbinate (OE)	0.2 (0–2.1)	0	34.5 (12.0–56.4)	1.3 (0–11.1)

^{a)} Nostril~Posterior edge of the upper incisor teeth.^{b)} ~Incisive papilla.^{c)} ~Anterior edge of the upper molar teeth.^{d)} luminant surface epithelium, RE ; respiratory epithelium, OE ; olfactory epithelium.^{e)} Average % and range from 10 studies.

Localization of respiratory metaplasia of the nasal gland epithelium is shown in **Table 2**. In both male and female rats, most lesions appeared in the nasal septum and nasoturbinate of level II. In contrast, the lesions in mice were distributed in the dorsal wall, nasal septum, and nasoturbinate or ethmoturbinate of levels I to III in males and in the dorsal wall, nasal septum and nasoturbinate of level I, and the nasal septum and nasoturbinate of level II in females. Variation among the studies was much greater in mice than in rat.

In rats, a few nasal glands beneath the respiratory epithelium were affected at the dorsal part of the nasal septum and/or nasoturbinate of level II in most cases (Fig. 1). Several nasal gland epithelia were replaced by ciliated columnar epithelial cells, and the number of normal nasal gland was decreased. Metaplastic glandular epithelium was often continuous to the luminal surface respiratory epithelium (Figs. 1 and 2), and the cytoplasm frequently contained eosinophilic globules (Fig. 3).

In mice, severe lesions were more widely distributed than in rats. Metaplastic glandular epithelium was mostly continuous to the luminal surface respiratory or olfactory epithelium, with a remarkably uneven surface (Figs. 4 and 5). In cases where Bowman's glands were affected, the olfactory epithelial cells were also metaplastic and the olfactory nerve bundles in the underlying lamina propria were atrophied (Fig. 6). In the glands of both the respiratory and olfactory regions, the cytoplasm of metaplastic epithelial cells sometimes contained eosinophilic globules.

Stratification and/or dysplasia of metaplastic cells was

not observed in either rats or mice.

The survival rates and the incidence of spontaneous lesions of animals used for this study varied, but were not in agreement with the incidence of respiratory metaplasia of the nasal gland epithelium. The distribution and severity of the lesions were not different between the animals sacrificed on schedule and those found dead or sacrificed in a moribund condition, and also for the housing and environment of animals and the type of food.

The present study revealed that the incidence, distribution, and histopathology of respiratory metaplastic lesions in the nasal subepithelial glands differed between rats and mice. In most male and female rats, the lesions were observed in the subepithelial glands at the olfactory and respiratory junction of the nasal septum and nasoturbinate of level II, including the luminal surface epithelium, as reported in cases of exposure to dimethylamine¹⁰. The distribution of nasal lesions is known to be greatly variable after inhalation of toxic materials¹⁸ because of the complexity of the nasal passages in laboratory animals and man^{19,20}, making it difficult to differentiate induced lesions from spontaneous ones.

In both male and female mice the incidence of lesions was significantly lower than that in rats, but the lesions were more widely distributed, extending to both the respiratory and olfactory regions, and varied among the studies. The incidence was higher and distribution was more extensive in males than females. A sex difference was particularly clear at the olfactory epithelium of level III. In our previous study² also, respiratory metaplasia of the olfactory epithelium was more severe in male mice than females and less frequent

Fig. 1. Respiratory metaplasia of the nasal gland epithelium at the olfactory and respiratory junction of the level II septum (S) and turbinate (T) of a male rat. The metaplastic glandular epithelium is often continuous to the luminal surface epithelium (arrows). HE stain. Bar = 100 μ m.

Fig. 4. Respiratory metaplasia of the nasal gland epithelium at the level I dorsal wall of a male mouse. The metaplastic glandular epithelium is frequently continuous to the remarkably uneven surface epithelium (arrows). S: nasal septum, T: turbinate. HE stain. Bar = 100 μ m.

Fig. 2. Nasal glands are replaced by the ciliated respiratory epithelium in the level II nasoturbinate of a male rat. The metaplastic glandular epithelium is often continuous to the luminal surface epithelium (arrows). HE stain. Bar = 40 μ m.

Fig. 5. Nasal glands replaced by the ciliated respiratory epithelium in the level I dorsal wall of a male mouse. The metaplastic glandular epithelium is often continuous to the luminal surface respiratory epithelium (arrows). HE stain. Bar = 40 μ m.

Fig. 3. Eosinophilic globules in the metaplastic glandular epithelium of the level II septum of a male rat (arrow). S: nasal septum, T: turbinate HE stain. Bar = 40 μ m.

Fig. 6. Respiratory metaplasia of the Bowman's glands at the level III dorsal wall of a male mouse. The metaplastic glandular epithelium is continuous to the luminal surface (arrows). The olfactory epithelial cells are also metaplastic (arrow heads). HE stain. Bar = 100 μ m.

in rats.

The normal nasal glands of lamina propria were decreased, and in those regions glandular structures were replaced by ciliated columnar epithelial cell. We have been using the term respiratory metaplasia of the nasal glands to describe this change. It was reported that squamous metaplasia of nasal glands occurred following the administration of certain toxic compounds such as 1,2-dibromo-3-chloropropane¹². These lesions were not observed as aging lesions in our studies. But respiratory metaplasia of the nasal glands presented as an aging lesion. Respiratory metaplasia of the glandular epithelium was thought to extend to the ductal epithelium of the subepithelial glands and then to the surface epithelium. Although the site specificity and particular pathogenesis of the lesions were not understood. These lesions were common in aged animals but not in young animals². The shape and capacity of the nasal cavity, area of the mucosal surface and air flow pattern have been found to be markedly changeable during the life span of rats and mice^{13,21,22}. However, species-, sex- and environment-dependent differences in the occurrence of the metaplastic lesions remain unclear.

Rodents, which are so frequently used for toxicologic studies, are obligate nose breathers and their sense of smell is dependent on breathing²³. In inhalation toxicity studies, special attention should be paid to the distribution and severity of spontaneous lesions in the nasal and upper respiratory tract, and it is importance to differentiate between such spontaneous lesions and those produced by toxin inhalation.

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