Sequential Changes in Tumor Development Induced by
1,4-Dinitrosopipperazine in the Nasal Cavity of F344 Rats

Tsuyoshi Takano, Tomoyuki Shirai, Tadashi Ogiso, Hiroyuki Tsuda, Shunkichi Baba, and Nobuyuki Ito

Abstract

Tumor development was examined sequentially in the nasal cavity of male F344 rats given 0.01% 1,4-dinitrosopiperazine (DNP) in their drinking water for various periods. Rats were sacrificed at 10-week intervals for up to Week 50. On continuous DNP administration, simple hyperplasia of the nasal cavity epithelium was observed from Week 10, papillary hyperplasia, nodular hyperplasia, and papilloma were observed from Week 20, and carcinoma was observed from Week 30. Carcinoma was observed in 100% of the animals given DNP for 50 weeks. The carcinomas were adenocarcinomas (86%), adenosquamous cell carcinomas (10%), and undifferentiated carcinomas (4%). Simple hyperplasia was distributed evenly in the nasal cavity. Seventy % of the nodular hyperplasias and 81% of the carcinomas were located in the ethmoturbinate and about 75% of the papillary hyperplasias and papillomas were located in the nasoturbinate and maxilloturbinate. These findings suggest that nodular hyperplasia is very important as a precursor of carcinoma in the nasal cavity of rats treated with DNP and that papilloma is less important in relation to carcinoma development.

Introduction

Occupational exposure to certain chemicals results in the appearance of carcinomas in the nasal or paranasal cavity (1). However, until recently, no experimental model of tumors of the nasal cavity was available, and the early developmental stages could not have been examined. Recently, some nitroso compounds (2, 5, 10-12) and other chemicals (3, 4, 9, 13) were found to induce tumors of the nasal cavity in various animals, thus providing suitable animal models of the disease in humans. However, thus far, there has been no report on the histogenesis of tumors of this organ.

Nitrosopipperazine and its homologs are potent carcinogens of the nasal cavity, upper digestive tract, and liver in rats, and nitrosamine and nitrosamino cell carcinomas (10%), and undifferentiated carcinomas (4%). Simple hyperplasia was distributed evenly in the nasal cavity. Seventy % of the nodular hyperplasias and 81% of the carcinomas were located in the ethmoturbinate and about 75% of the papillary hyperplasias and papillomas were located in the nasoturbinate and maxilloturbinate. These findings suggest that nodular hyperplasia is very important as a precursor of carcinoma in the nasal cavity of rats treated with DNP and that papilloma is less important in relation to carcinoma development.

Results

The rats that were given DNP grew more slowly than did untreated controls, and the decrease in their mean body weight was proportional to the period of treatment. At the end of the experiment, animals that were given DNP for 50 weeks weighed 80% as much as the controls.

One of the earliest signs of tumor formation was bilateral or unilateral eye discharge (Fig. 1). This was followed frequently by localized swelling over the nasal bones, which was observed first in rats given DNP continuously for 36 weeks. Stridor or dyspnea was often seen. Occasionally, bloody discharge from the nose was also observed, and some rats died from massive bleeding from the external nose hole.

Macroscopically, grayish tumor masses were found in the side wall of the nasal cavity of many animals. Tumors were multiple in many cases. In some cases, a big tumor developed in the nasal cavity resulting in separtal deformation. Invasive growth of tumors into the surrounding tissue was noted in advanced cases.

Histologically, lesions of the nasal cavity epithelium were classified into 5 types: simple hyperplasia; papillary hyperplasia; papilloma; nodular hyperplasia; and carcinoma.
Sequential Changes to Tumor in the Nasal Cavity

Simple hyperplasia consisted of focal thickening of the mucosa with 6 to 10 layers of epithelial cells (Fig. 2). Ciliated cells were no longer seen at the lesion, and cells showed slight irregularities. Papillary hyperplasia was an upward growth protruding into the nasal cavity with a delicate fibrovascular core (Fig. 3). In most cases, the changes were strictly localized. No cellular atypism or mitotic figures were seen. Nodular hyperplasia was a downward growth of the epithelium into the submucosal area, and in most cases the changes were localized (Fig. 4). Nodular hyperplasia occasionally had glandular formation, in which periodic acid-Schiff-positive material was seen. These changes were called "inverted papilloma" by Norris (7). Papilloma was defined as a benign epithelial tumor in which the epithelial cells were arranged in branched finger-like processes with fibrovascular cores (Fig. 5). Cellular irregularity was slight, and few mitotic figures were present. Carcinomas showed characteristics such as anaplasia and invasion. From their histological patterns, the carcinomas were classified into 3 types: adenocarcinomas (Fig. 6); adenosquamous carcinomas (Fig. 7); and undifferentiated carcinomas (Fig. 8).

The incidences of these epithelial lesions induced by DNP are shown in Table 1. Sequential changes in the incidences of lesions in rats treated with DNP for periods of 10 weeks (Group 1) and 50 weeks are shown in Chart 2. The latter data represent results in Groups 1 to 5. The earliest lesions were simple hyperplasias, and these were followed by papillary or nodular hyperplasias. In Group 1, in which rats were treated with DNP for 10 weeks, simple hyperplasia, papillary hyperplasia, nodular hyperplasia, and papilloma appeared first in Weeks 10, 20, and 30, respectively. Carcinoma was first noted in Week 50.

Table 1

<table>
<thead>
<tr>
<th>Experimental period (wk)</th>
<th>No. of rats with Simple hyperplasia</th>
<th>Papillary hyperplasia</th>
<th>Nodular hyperplasia</th>
<th>Papilloma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Total</td>
<td></td>
<td>10/0</td>
<td>10/0</td>
<td>10/0</td>
<td>10/0</td>
</tr>
<tr>
<td>10</td>
<td>10/0/8</td>
<td>1 (13)*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>10/10/9</td>
<td>4 (44)</td>
<td>1 (11)</td>
<td>2 (22)</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>10/20/8</td>
<td>7 (88)</td>
<td>4 (40)</td>
<td>2 (25)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>40</td>
<td>10/30/7</td>
<td>7 (100)</td>
<td>4 (57)</td>
<td>7 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>50</td>
<td>10/40/12</td>
<td>11 (92)</td>
<td>5 (42)</td>
<td>10 (83)</td>
<td>6 (57)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.
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hyperplasia

ages of the total number of lesions. Carcinomas in this chart do not include hyperplasia

4 regions: nasoturbinate (i); ethmoturbinate (•); respiratory

Papilloma

Nodular _

Papillary

Simple

tum; and olfactory septum. The 5 epithelial lesions were scored according to their original site and distribution as shown in Chart 3. Simple hyperplasia (total number, 213) was distributed approximately evenly in all sites of the nasal cavity. Papillary hyperplasia (121 cases) and papilloma (196 cases) showed quite similar distributions. About 75% of these lesions occurred in the nasoturbinate and maxilloturbinate, and the remaining 25% were evenly distributed in the other 3 regions. Nodular hyperplasia was located most often in the ethmoturbinate (149 of 213 cases, 70%). Of the carcinomas, about 62% were so widely invasive that their primary site could not be determined. Of the carcinomas in which the primary site was clear, 81% were located in the ethmoturbinate, 11% were found in the nasoturbinate and maxilloturbinate, 4% were located in the respiratory septum, and 4% were found in the olfactory septum. This distribution pattern was similar to that of nodular hyperplasia.

Incidence (%)

0 50 100

Simple hyperplasia

Papillary hyperplasia

Nodular hyperplasia

Papilloma

Carcinoma

Chart 3. Distribution of nasal cavity tumors. The nasal cavity was divided into 4 regions: nasoturbinate and maxilloturbinate (□); ethmoturbinate (○); respiratory septum (△); and olfactory septum (●). Symbos indicate incidences (%) of lesions in each region. Incidences of lesions in each region were calculated as percentages of the total number of lesions. Carcinomas in this chart do not include widely invasive ones.

DISCUSSION

The present study confirms essentially the carcinogenic effect of DNP on the nasal cavity of rats reported by Reznik et al. (8) and Lijinsky and Taylor (5). Many other chemical carcinogens induce tumors in the nasal cavity of animals. Although early changes that may precede to carcinoma of the nasal cavity were reported in some works (9, 13), no sequential analysis of the carcinogenic process in this organ has been reported.

Under the present experimental conditions, we were able to distinguish 5 different proliferative lesions: simple hyperplasia; papillary hyperplasia; nodular hyperplasia; papilloma; and carcinoma. Sequential analysis of tumor development showed that simple hyperplasia was the earliest lesion, followed by papillary hyperplasia, nodular hyperplasia, papilloma, and finally, after a slight delay, carcinoma. These lesions appeared successively in rats given DNP for 10 weeks, but the incidences of all of them except carcinoma had decreased by Week 50. On continuous administration of DNP, the incidence of nodular hyperplasia was decreased in Weeks 40 and 50. This decrease in their incidences in later stages of the experiment was probably due to an increase in the incidence and size of advanced lesions.

Although it was not easy to see from sequential observations how the 5 different lesions are related to each other, their clear relationship was disclosed from their characteristic localizations in the nasal cavity. Papillary hyperplasia and papilloma both tended to be located in the nasoturbinate and maxilloturbinate (75% of both lesions), whereas nodular hyperplasia and carcinoma were located mainly in the ethmoturbinate (70% of the former and 81% of the latter). Furthermore, these 2 pairs of lesions often coexisted in the respective regions. These findings suggest that papillary hyperplasia progresses to papilloma and that nodular hyperplasia progresses to carcinoma. It is unlikely that papilloma progresses to carcinoma. In the nasal cavity of humans, nasal or paranasal papillomas are well known to be preneoplastic changes. From their growth form, papillomas are classified into 2 types, exophytic and inverted (6, 7). The papillomas and nodular hyperplasias seen in this study were similar to human exophytic papillomas and inverted papillomas, respectively. It is said that, in humans, inverted papillomas may give rise to carcinomas (7), and the present results support this idea. The relationship between simple hyperplasia and papillary or nodular hyperplasia is unclear. It is likely that some areas of simple hyperplasia progress into papillary or nodular hyperplasia, especially into the latter (Figs. 2 and 4). However, we cannot rule out the idea that papillary or nodular hyperplasia arise de novo.

Squamous cell metaplasia has been suggested to be a precursor in the carcinogenic process of the respiratory tract. In the nasal cavity, squamous cell metaplasia has been reported in humans (14) and in experimental animals (8, 9, 13). In the present experiment with DNP, the incidence of squamous cell metaplasia of the nasal cavity epithelium was lower than that found in other studies (8, 9, 13). This low incidence of squamous cell metaplasia in hyperplasia and papilloma may be related to the absence of squamous cell carcinoma in the present study. Squamous cell elements in carcinomas were always observed as mixtures with adenocarcinoma cells. Although most malignant tumors in the human nasal or paranasal...
cavity are squamous cell carcinomas (14), it seems probable that the histological pattern of tumors in the nasal cavity varies in different species of animals and with the carcinogen used. Most of the malignant tumors in the present experiment were adenocarcinomas. The tumors induced in Sprague-Dawley rats by DNP were of the squamous cell type (8). Inhalation of formaldehyde induced squamous cell carcinomas but not adenocarcinomas in F344 rats (13). Adenocarcinomas developed in more than 70% of the hamsters given N-nitroso-2,6-dimethylmorpholine s.c. (2).

The present study suggests that nodular hyperplasia is particularly important as a precursor of carcinoma and that papilloma is probably less important in terms of cancerization. Studies on histogenesis in the nasal cavity carcinogenesis with DNP should be of value in providing guides for assessing the prognosis and suitable therapy of tumors of the nasal cavity in humans.

REFERENCES


Fig. 1. Eye discharge and swelling around the nose of a rat given DNP for 50 weeks.
Fig. 2. Simple hyperplasia of the nasal cavity epithelium in a rat given DNP for 20 weeks and then killed. H & E, x 200.
Fig. 3. Papillary hyperplasia of the nasal cavity epithelium of a rat treated with DNP for 20 weeks and then killed. H & E, x 100.
Fig. 4. Nodular hyperplasia of the nasal cavity epithelium of a rat treated with DNP for 30 weeks and then killed. H & E, x 200.
Fig. 5. Papilloma of the nasal cavity of a rat given DNP for 30 weeks and then killed. H & E, x 100.
Fig. 6. Adenocarcinoma of the nasal cavity in a rat given DNP for 40 weeks and then sacrificed. H & E, x 200.
Fig. 7. Adenosquamous carcinoma of the nasal cavity of a rat given DNP for 40 weeks and then sacrificed. H & E, x 200.
Fig. 8. Undifferentiated carcinoma of the nasal cavity of a rat given DNP for 40 weeks and then sacrificed. H & E, x 100.
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