Tumorigenesis in the Nasal Olfactory Region of Syrian Golden Hamsters as a Result of Di-n-Propylnitrosamine and Related Compounds

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SUMMARY

The morphology of neoplasms in the nasal olfactory region of the Syrian golden hamster observed after s.c. injections of di-n-propylnitrosamine, β-hydroxypropyl-n-propylnitrosamine, β-oxopropyl-n-propylnitrosamine, and methyl-n-propylnitrosamine are described; all four compounds induced tumors. Neoplastic lesions were characterized by the proliferation of three often intermingled cell types (large cuboidal, cylindrical, and small cell). These cells were similar to cells of olfactory glands, sustentacular cells, and basal cells. Neurogenic elements were not identified in the tumors. Therefore these neoplasms were called carcinomas originating in the olfactory epithelium; the diagnostic criteria were discussed.

INTRODUCTION

The majority of malignant tumors in the nasal cavity in man are carcinomas (4, 9, 31) and only rarely esthesioneuromas (5, 19, 26). Occupational exposure to certain chemicals results in the appearance of carcinomas in the nasal cavity (1, 7, 13, 21). Laboratory animals frequently developed esthesioneuroepitheliomas induced by various nitroso compounds (30); Syrian golden hamsters were reported to show these tumors after application of diethylnitrosamine and dimethylnitrosamine (15, 16). The next higher homolog of diethylnitrosamine, DPN, and compounds related to its β-oxidation, HPPN, OPPN, and MPN, induced tumors in all parts of the respiratory system of Syrian golden hamsters (2) including the olfactory region of the nasal cavity. The morphology of neoplasms found in the olfactory region of the nasal cavity is the subject of the present report.

MATERIALS AND METHODS

Six- to 8-week-old Syrian golden hamsters from the Eppley colony were housed in plastic cages in groups of 5 according to sex. They received a pellet diet and water ad libitum and were kept under standardized conditions.

DPN, HPPN, OPPN, and MPN dissolved in olive oil were administered s.c. once weekly for life at the equitoxic doses related to the LD₅₀: DPN at 3.75, 7.5, 15, 30, and 60 mg/kg body weight to 20 females and 20 males per dose level; HPPN at 37.5, 75, and 150 mg/kg body weight to 10 females and 10 males per dose level; OPPN at 30, 60, and 120 mg/kg body weight to 10 females and 10 males per dose level; and MPN at 12.5, 25, and 50 mg/kg body weight to 10 females and 10 males per dose level. Fifty females and 50 males receiving only the solvent served as controls.

Animals were sacrificed when moribund. Skulls were fixed in 10% buffered formalin and decalcified in Decal, and the vertical sections of the nasal cavity were embedded in Paraplast. Step and, if necessary, serial actions were stained with hematoxylin and eosin, PAS, Alcian blue, Kreyberg, Luxol-fast blue, phosphotungstic acid-hematoxylin, Bodian's, and Gomori's reticulum stain.

RESULTS

The number of animals, average survival, incidence of tumors in the olfactory region, and latency period of these neoplasms according to compound, dose, and sex are given in Table 1.

Olfactory Epithelium. In the Syrian golden hamster, the yellowish epithelium of the olfactory region covers a small portion of the roof of the nasal cavity, the neighboring parts of the nasal septum in the anterior region, and the entire posterior region of the cavities including parts of the nasoturbinals and ethmoturbinals. Microscopically, the olfactory mucosa consists of a superficial epithelium and a tunica propria separated by a basal lamina. The pseudostratified and columnar epithelium is composed of sensory cells, columnar supporting sustentacular cells, and small basal cells arranged in definite zones (Fig. 1). The irregularly shaped small basal cells rest on the basal lamina. The bipolar sensory cells are situated at different levels in the middle layers of the epithelium and form an extensive zone of cells with round nuclei with an “owl eye” appearance, which is due to a prominent central basophilic nucleolus and the delicate chromatin granules attached to the nuclear membrane. The more slender and taller sustentacular cells line the surface of the epithelium. Their nuclei, generally oval with finely dispersed chromatin, lie in a
Table 1

Tumor induction in the olfactory region of the Syrian golden hamster according to different dose levels of compounds

<table>
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<tr>
<th>Dose (mg/kg)</th>
<th>Effective no. of animals</th>
<th>Av. survival (wk)</th>
<th>Tumor-bearing animals</th>
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The 1st alteration observed in Bowman's glands consisted of enlargement of cells; in some cells clear areas beyond the round or oval nuclei (Fig. 2) were seen. The ducts were distended, forming secondary glandular structures within the epithelial layer (Fig. 2). These changes were followed by proliferation of cells which showed abundant eosinophilic cytoplasm with occasional PAS- and Alcian blue-positive droplets and "vacuoles"; in other areas the cells were columnar with transparent cytoplasm containing brush-like projections from the free surface into the glandular lumen (Fig. 8). These cells resembled sustentacular cells. In some instances the cells were relatively smaller than those usually seen; they showed peripherally placed round, hyperchromatic, closely packed, garland-like arrangements (Fig. 10).

The hyperplasia of the glandular cells was frequently associated with concomitant proliferation of the superficial epithelium (Fig. 7). In such instances cytologically similar cells lined both the luminar surface and the glands (Figs. 7 to 11). In the lumen of the glands as well as in periglandular spaces, PAS-negative, round droplets similar to those seen in the proliferated superficial epithelium (Fig. 6) were present (Figs. 9, 11, and 12). In some instances, the proliferated glands and occasionally also the superficial epithelium showed squamous cell metaplasia (Fig. 12).

Alterations in Advanced Stages of Tumor Development. In animals surviving longer and particularly in those receiving the highest doses of carcinogens the neoplastic lesions were exophytic showing pleomorphism and cell atypia. In the most advanced stages the tumors almost completely filled the corresponding side of the nasoturbinals, destroying them by
invasion and extending to the anterior part of the nasal cavity; some tumors invaded the ethmoturbinals (Fig. 13A) and the corresponding sides of the brain. The tumors were multifocal and of various histological patterns (Figs. 13B and 14 to 16).

Large cuboidal cells resembling the cells of a Bowman’s gland were arranged, even in the same tumor, in different patterns. Glandular formations were lined by eosinophilic cuboidal cells (Figs. 14 to 16) containing PAS- and Alcian blue-positive mucus droplets. These cells showed patterns sometimes similar to oncocytes (Fig. 24). In other areas, including neoplasms invading the brain, they formed cystic structures, showing fragmentation of the cytoplasm suggesting secretion (Fig. 17). In other regions, papillary formation (Fig. 18), mixed carcinomatous and sarcomatous structures (Fig. 19A), or predominantly sarcomatous patterns (Figs. 19B and 20A) with areas of ossification (Fig. 20B), hyalin, or myxoid degenerations were encountered.

Cylindrical cells partially resembling sustentacular cells were frequently arranged in a glandular pattern in which cells had transparent cytoplasm (Fig. 16), occasionally with brush-like projections into the lumen. In other areas the cells were relatively small with hyperchromatic and fairly uniform nuclei. They were particularly seen in areas of brain invasion where they showed focal transition to large atypical cell forms (Fig. 21). In some instances focal squamous cell metaplasia was present. “Rosette” or “pseudorosette” formations (Fig. 22) were also common.

Small cells consisting of small, round, or oval cells with centrally located dense nuclei and a scantly halo or light eosinophilic cytoplasm and ill-defined cell borders were frequently found in parts of the tumors (Figs. 14 and 15). They infiltrated the lamina propria (Fig. 15) and were usually closely packed. In some instances, the presence of fusiform dense nuclei and the arrangement of the cells (Fig. 26A) caused them to resemble “oat cell carcinoma.” Occasionally, they formed solid nests with palisade-like arrangement of nuclei at the periphery, thus resembling “basal cell carcinoma” (Fig. 23). Focal hyalin droplets and myxoid material were present. The cells also formed cystic spaces, particularly in the brain, showing squamous cell differentiation often with keratinization (Figs. 26B, 27, and 28A and B). In other areas they portrayed a mixture of glandular and rosette or pseudorosette formations (for definitions see Ref. 27) thus merging into tubular and duct structures (Fig. 25) or into squamous cell formations (Figs. 26B and 27).

The rosettes and pseudorosettes seen in tumors of both cylindrical and small cell types were formed by cylindrical or carrot-like inwardly directed cells with slightly eosinophilic cytoplasm, containing 1 or more layers of peripherally located oval or round dense nuclei. The lumina in rosettes were tiny, distinct, or ill defined. They contained pyknotic nuclei towards the lumen and also occasional mitotic figures (Figs. 22 and 25). PAS, Alcian blue, and Kreyberg staining showed mucus in the lumina of the rosettes and droplets in the apical part of the cytoplasm. Occasionally, keratin pearls were present in the lumens of the rosettes, predominantly of the small cell type (Fig. 26B). Neurofibrils were not found in the tumors by the described methods. In the type of pseudorosette formation resulting in perpendicular palisading of cells around the vessels, the nuclei usually faced the vessel wall (Figs. 22 and 25). In perivascular spaces, as well as in the lumina of glandular structures and rosettes, either clear serous material or conspicuously uniform, round PAS-negative granules were frequent (Fig. 25).

These tumors were composed of all 3 cell types (Figs. 14 to 16 and 24). There was no remarkable preference of combinations; even in areas with a predominance of 1 cell type, small foci of other cell types were present (Fig. 24). Proliferation of neither sensory epithelial cells nor nerve fibers could be observed.

DISCUSSION

Sensory cells of the olfactory epithelium seem to be most sensitive to toxins. Selective acute degeneration and necrosis of these cells have been shown after administration of di-N-methylaminoethyl nitrosamine and particularly of di-N-ethylaminoethyl nitrosamine to Syrian golden hamsters (12). The latter compound is a potent carcinogen for the respiratory tract (nasal cavity included) in this animal (15, 20). The high sensitivity of the sensory cells of the olfactory epithelium to the toxic effect of carcinogenic nitrosamine compounds was also demonstrated in the present study as seen in degenerative change, atrophy, and disappearance of the sensory cells. Proliferation of these cells was not observed. Apparently, they have no part in the early and later tumor development, as they do not regenerate under the described experimental conditions.

Dysplasia and hyperplasia preceded tumor development as seen in several tissues (22) but were also seen simultaneously with tumors. The large cuboidal, cylindrical, and small cells forming the tumors showed similarities to the cells of Bowman’s gland, and to the sustentacular and basal cells of the olfactory epithelium. The absence of neurogenic cells in the tumors may be explained by the assumption that the transformed basal cells apparently do not develop into this highly differentiated and functionally specialized cell type (10). However, the concomitant presence of the cells similar to those of Bowman’s gland, sustentacular, and basal cells and transitional forms in the proliferative and neoplastic stages suggest the origin of these cells from the epithelial component of the olfactory epithelium (3, 11). Tumors of the small cell type which resembled those of bronchial “oat cell tumors” or “basal cell carcinoma” of skin showed a tendency toward glandular, rosette, and pseudorosette formation and also to squamous cell differentiation. This tendency of multiple differentiation has also been reported in carcinomas of the respiratory tract in man (31). Rosettes and pseudorosettes observed in the tumors of the present investigation are of particular interest since similar features are common in neurogenic tumors (18, 19, 27, 28, 32, 33). They were also described in esthesioneuroepithelial tumors in hamsters after di-N-methylaminoethyl nitrosamine and di-N-ethylaminoethyl nitrosamine treatment (15, 16).

Esthesioneuroepitheliomas are defined by the presence of 2 components, epithelial and neurogenic elements (19, 26). However, in the present study, only epithelial elements were found in the neoplasms showing secretory activity, squamous cell metaplasia, and keratin formation. Rosette formation as also seen in the described tumors is apparently not characteris-
tic of neurogenic tumors since similar structures have been seen in mesenchymal (23) and other epithelial tumors (9, 14, 31), particularly in metastasis of bronchogenic oat cell carcinoma into the brain (27). Pseudorosettes, as usually found in tumors of neurogenic origin showing cell arrangements around a vessel with peripherally located nuclei, were not observed in the present tumors; however, perivascular cell arrangements were frequently seen, in which the nuclei of the tumor cells usually faced the vessel wall. In the perivascular spaces of these pseudorosettes, serous material or round, strikingly uniform PAS-negative granules were frequent. These granules were also observed in glandular structures during the proliferative stage of the olfactory epithelium. They are not characteristic of neurogenic tumors. Other marked differences were the lack of neurofibrils and blepharoplasts in tumors in the present study and the tendency of the tumor cells toward squamous cell differentiation even in invasion into the brain. Therefore, the possibility that preexisting squamous cell epithelium surrounded the tumor cells (30) was excluded. From these findings it can be concluded that these tumors in the olfactory region of hamsters seen under the described conditions correspond to carcinomas.

Carcinomas were also reported in the nasal cavity of Syrian golden hamsters after di-α-ethyl-nitrosamine treatment (20, 29). They were mainly found in the anterior respiratory region (20) and were also seen in the posterior olfactory region (29); anaplastic carcinomas derived from both the respiratory and the olfactory regions (20). Transplacental administration of nitrosourea compounds induced a variety of neural neoplasms, but no tumors of the olfactory bulbs or nerves were reported in the laboratory animals (8, 33). Tumors in the olfactory region described in these investigations may add to the information on the comparative morphology and histogenesis of "spon-

ACKNOWLEDGEMENTS

We thank Professor K. J. Zülch, Max-Plank-Institute für Hirnforschung, Köln, Germany, for his critical advice; Dr. F. W. Krüger for synthesizing the compounds; A. Washington and W. Williams for photography; Donna Janecek for editorial assistance; and Adi Guldimann for skilled technical assistance.

REFERENCES


Fig. 1. Olfactory epithelium in untreated hamster. a, sustentacular cells; b, sensory cells; c, basal cells; d, Bowman's gland. H & E, × 300.

Fig. 2. Epithelium of olfactory region in a treated hamster showing replacement of sensory cells by sustentacular and small cells. In addition, enlargement of cells and distention of Bowman's glands are present. H & E, × 375.

Fig. 3. Focal squamous cell metaplasia of the superficial epithelium in ethmoidal region. H & E, × 300.

Fig. 4. Papillary proliferation of the superficial epithelium; the cells represent a transition between sustentacular cells and cells of Bowman's gland. H & E, × 175.

Fig. 5. Intraepithelial glandular formation of sustentacular cell type. H & E, × 400.

Fig. 6. Nodular hyperplasia of small cell with intraepithelial glandular formation. Round granules are present in the lumina and on the free surface. H & E, × 225.

Fig. 7. Alteration of Bowman's glands of olfactory mucosa in early stages of tumor development. Proliferation of large cuboidal cells forming both the surface and the glands. H & E, × 200.

Fig. 8. Cylindrical cells with brush-like projections lining the lumen of the nasal cavity and the glands. H & E, × 180.

Fig. 9. Cylindrical cells with autolytic alterations. The same epithelium covering both the glands and the surface is in the lower part sloughed.

Fig. 10. Cells with scanty cytoplasm and garland-like arrangement of nuclei. H & E, × 160.

Fig. 11. Different types of cylindrical cells on the surface merging into glands; some small cells with clear halos are also present (arrows). H & E, × 200.

Fig. 12. Squamous cell metaplasia in superficial epithelium (lower left) and in proliferated glands. H & E, × 200. Inset, another area of these alterations and brush-like projections into the lumen. H & E, × 250.

Fig. 13. Tumors in the posterior part of the nasal cavity. A, macroscopic appearance of tumors; B, histological section of the same specimen. The corresponding sections of tumors (a, b, c) are illustrated in Figs. 14 to 16.

Fig. 14. Tumor consisting of large cuboidal cells (right) and small cells (left) showing round or fusiform nuclei. H & E, × 100.

Fig. 15. Small cell tumor with carrot-like or fusiform dense nuclei resembling basal cell carcinoma. Right half, tumor of large cuboidal cell type. H & E, × 100.

Fig. 16. Combination of large cuboidal and cylindrical cells. H & E, × 100.

Figs. 17 to 20. Tumors of the large cuboidal cell type.

Fig. 17. A tumor invading the brain shows cystic formation of large cuboidal cell type. H & E, × 80. Fragmentation of supranuclear cytoplasm is more obvious at higher magnification (Inset, × 200).

Fig. 18. Papillary structures of a tumor with large cuboidal cells. H & E, × 90.

Fig. 19. Mixture of glandular and sarcomatous pattern (A) and sarcomatous structure (B). H & E, × 100.

Fig. 20. Pleomorphic atypical cells (A) merging in sarcomatous structures showing focal ossification (B). H & E, × 250.

Fig. 21. Tumors of a lesion showing uniform round hyperchromatic nuclei infiltrating the olfactory bulb. In another area of the same tumor focal transition to large cells is shown (Inset). H & E, × 200.

Fig. 22. Cylindrical cells forming rosettes and pseudorosettes. H & E, × 180. Mitotic figures and pyknotic nuclei in the luminal part of the cells (Inset, × 420).

Fig. 23. Small cells with solid structures resembling basal cell carcinoma. H & E, × 150.

Fig. 24. Mixture of small and large cuboidal cells; in the middle focal clear cell formation. H & E, × 110. Inset, pleomorphism of small cells and phagocytosis.

Figs. 25 to 28. Tumors of small cell type.

Fig. 25. Round granules in perivascular spaces. H & E, × 300. Rosette and pseudorosette merging in tubular glandular structures in the same tumor (Inset).

Fig. 26. A mixture of rosette, pseudorosette, and streaming of cells. H & E, × 200. B, another area of the same tumor showing keratin (Kreyberg red) in the lumen of rosettes. H & E, × 225.

Fig. 27. Small cells with transformation to keratinizing cells. Kreyberg, × 200.

Fig. 28. Invasion of the tumor illustrated in Fig. 26 into the brain, showing squamous cell differentiation with keratinization. H & E, × 95. B keratinizing squamous cell metaplasia of the tumor (Fig. 27) after invasion into the brain. H & E, × 95. Inset, squamous cell differentiation. H & E, × 210.
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JANUARY 1974
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