Molecular Dosimetry of DNA Adduct Formation and Cell Toxicity in Rat Nasal Mucosa following Exposure to the Tobacco Specific Nitrosamine 4-(N-Methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone and Their Relationship to Induction of Neoplasia

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ABSTRACT

The molecular dosimetry of O6-methylguanine (O6MG) formation in DNA and cytotoxicity in respiratory and olfactory mucosa was determined during administration of 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to male Fischer 344 rats. The dose response for O6MG formation differed considerably between respiratory and olfactory mucosa. The dose response was nonlinear in respiratory mucosa where the concentration of O6MG formed to dose of NNK ratio, an index of efficiency of alkylation, increased dramatically only in the respiratory mucosa as the dose of NNK was decreased from 100 to 0.3 mg/kg. The concentration of O6MG was four times greater in respiratory than olfactory mucosa after treatment of rats with 1 mg/kg NNK. Alkylation in the two regions of the nose became similar as the dose of NNK was increased. In rats treated for up to 12 days with NNK (10 mg/kg/day), the concentration of O6MG was 60 to 90% greater in respiratory than olfactory mucosa throughout treatment.

The molecular dosimetry of 06-methylguanine-DNA-methyltransferase activity was found to decrease with a time course similar to the loss of O6MG observed during administration of carcinogen. The decrease in O6MG concentration appeared to be the result of cytotoxicity induced by exposure to NNK. Following only 4 days of treatment, severe necrosis of the olfactory mucosa and early metaplasia of the respiratory mucosa was evident. These data suggested that the marked susceptibility of the nose to NNK-induced tumor formation may stem from both the high concentration of O6MG and cell replication associated with toxicity induced by chronic exposure to this carcinogen (4). Since this earlier study utilized doses of NNK which were higher than those tested in carcinogenicity studies (2), it is difficult to assess the contribution of alkylation and cytotoxicity to tumor formation after low dose exposures.

INTRODUCTION

The tobacco specific carcinogens NNK2 and NNN are major carcinogenic constituents of tobacco products (1). Carcinogenicity studies with Fischer 344 rats have demonstrated that NNN induces tumors primarily in the nasal cavity while exposure to NNK is associated with a high incidence of lung, liver, and nasal cavity tumors (2). The potent carcinogenicity of NNK may stem in part from its metabolic activation via α-hydroxylation to a methylating agent (3).

Previously, the formation and removal of DNA adducts was examined in liver, lung, and nasal epithelium during multiple dose administration of NNK (100 mg/kg/day) to male Fischer 344 rats (4). After 1 day of treatment with NNK, the concentration of the promutagenic adduct O6MG was greatest in nasal mucosa followed by liver and lung. However, during the next 5 days of treatment, alkylation of DNA in nasal epithelium declined to one half the initial amount and remained constant for the remaining 6 days of carcinogen administration. The decrease in alkylation could not be explained by an increase in rates of adduct removal since O6-methylguanine-DNA-methyltransferase activity was found to decrease with a time course similar to the loss of O6MG observed during administration of carcinogen. The decrease in O6MG concentration appeared to be the result of cytotoxicity induced by exposure to NNK. Following only 4 days of treatment, severe necrosis of the olfactory mucosa and early metaplasia of the respiratory mucosa was evident. These data suggested that the marked susceptibility of the nose to NNK-induced tumor formation may stem from both the high concentration of O6MG and cell replication associated with toxicity induced by chronic exposure to this carcinogen (4). Since this earlier study utilized doses of NNK which were higher than those tested in carcinogenicity studies (2), it is difficult to assess the contribution of alkylation and cytotoxicity to tumor formation after low dose exposures.

The purpose of the present study was to determine the dose response for O6MG formation and cell toxicity in respiratory and olfactory mucosa following exposure to NNK at doses similar to and lower than those used in carcinogenicity studies.

MATERIALS AND METHODS

Carcinogen Treatment. NNK was synthesized (5, 6) by Chemsyn Science Laboratories (Lenexa, KS). Purity was greater than 99% according to nuclear magnetic resonance, ultraviolet, infrared, and thin layer chromatography. Male Fischer 344 rats (175–200 g; Charles River Breeding Laboratories, Kingston, NY) were treated with NNK (0.3, 1.0, 3.0, 10, 30, or 100 mg/kg, i.p.) dissolved in trioctanoin and sacrificed 4 h after carcinogen administration. In some experiments, rats were treated for up to 12 days with doses of NNK ranging from 1 to 100 mg/kg/day and killed over the course of the 12-day treatment period.

Carcinogenicity Study. The incidence and localization of neoplasia in...
the nose induced by chronic exposure to NNK was determined in a
carcinogenicity study employing 85 six-week-old Male Fischer 344 rats.
Sixty-eight rats received NNK (50 mg/kg, s.c.) dissolved in triacetin (0.05 mmol/0.25 ml triacetoin) 3 times a week for 20 weeks while 17 rats were treated with vehicle (trioctanoin) following the same dosing
regimen. The total dose of NNK was approximately 750 mg (3.75
mmol). Nine weeks after treatment was discontinued, six treated and
two control rats were sacrificed and nasal passages fixed in neutral
buffered formalin (10%) for histological examination. The remaining
animals were killed over a 35-week period following treatment. Rats
were killed when they exhibited a 10 to 15% loss of maximum body
weight within a 2-week period.

Histological Procedures. Nasal passages of rats from the carcinogenic-
ity study and from animals which had been treated (i.p. administr-
action) with 1, 10, 30, or 100 mg/kg NNK for 2, 4, or 12 days were
removed, fixed in neutral buffered formalin (10%), decalcified in 10%
formic acid for 3 days, and embedded in paraffin or glycol methacrylate.
Tissue sections were stained with hematoxylin and eosin or toluidine
blue for histopathological examination. Cytotoxicity was evaluated by
grading the degree of necrosis or metaplasia from 1+ to 4+, with 1+
indicating mild necrosis and 4+ representing either severe necrosis or
total replacement of normal epithelium by metaplastic basal cells.

Autoradiography. Three rats received (i.p. injection of 1.4 mCi/rat)
1 mg/kg [3H]NNK (Chemsyn Science) labeled on the N-methyl carbon
(specific activity, 1.4 Ci/mmol). Four h after treatment, the rats were
sacrificed and the nasal passages fixed with neutral buffered formalin.
Tissues were subjected to several washes in formalin until unbound
radioactivity reached background levels. The nondecalcified noses were
embedded in a mixture of glycol (50%) and methyl (50%) methacrylate.
2 μm sections were cut and mounted on glass slides. The slides were
dipped in NTB-2 emulsion (Eastman Kodak, Rochester, NY) and
exposed for 6 weeks, then developed in D19 (Eastman Kodak) developer
and coverslipped.

Microdissection and DNA Isolation. Rats were anesthetized with
Nembutal (Abbott Laboratories, Chicago, IL) and sacrificed for micro-
dissection of respiratory and olfactory mucosa from the nasal passages.
Respiratory mucosa was obtained from the naso- and maxilloturbinites,
lateral walls, and median septum anterior to the olfactory epithelium.
Olfactory mucosa was obtained from the ethmoturbinates, and the
lateral wall and medium septum of the olfactory area. Respiratory or
olfactory mucosa were pooled from two rats prior to DNA isolation.

DNA was isolated by digestion of respiratory or olfactory mucosa with
pronase (10 mg/ml) in 1% sodium dodecyl sulfate followed by
phenol-chloroform extraction and ethanol precipitation (7). Samples
were incubated with Rnase T (100 U) and Rnase A (300 U) for 3 h at
37°C and DNA recovered by ethanol precipitation. This procedure
removed all RNA contamination as detected by electrophoresis of DNA
on agarose gels.

Determination of Methylated Purines. The concentration of O6MG and
7MG in DNA from respiratory and olfactory mucosa from rats treated
with 3, 10, 30, or 100 mg/kg NNK was determined by fluores-
cence-linked HPLC. DNA was depurinated by heating in 0.1 M HCl
for 30 min at 80°C. The concentration of unmodified and methylated
purines was determined after HPLC separation and integration of UV-
absorbing and fluorescing peaks as previously described (8, 9). Separations
were achieved by using two strong cation exchange HPLC col-
umns (Partisil 10-SCX; Whatman, Clifton, NJ, 25 x 0.45 cm) in series.
O6MG and 7MG concentrations in DNA were determined in separate
HPLC runs. O6MG was separated from unmodified bases using an
isocratic gradient of 75 mM NH4H2PO4 + 11% methanol, pH 2.5, at a
flow rate of 1.5 ml/min. 7MG separation was achieved using an
isocratic gradient of 75 mM NH4H2PO4 + 1% methanol at a flow rate
of 1.5 ml/min. Limits of detection for 7MG and O6MG were 25 and
1.5 pmol/μmol of unmodified base, respectively.

A competitive radioimmunoassay was employed for the measure-
ment of O6-methyldeoxyguanosine in nasal mucosa from rats treated
with 0.3, 1, or 3 mg/kg NNK. The antibody to O6-methyldeoxyguano-
sine (10) was a gift from Dr. Roy Saffhill. DNA was enzymatically
digested to nucleosides using DNase I, alkaline phosphatase, and snake
venom phosphodiesterase as previously described (10, 11). Fifty µl were
removed from each sample and subjected to reverse phase HPLC with
integration of UV absorbing peaks to determine the concentration of
normal nucleosides (11). Digested DNA samples (0.2–0.4 mg) were
chromatographed using reversed phase HPLC and fractions collected,
dried under reduced pressure, and then subjected to radioimmunoassay
to determine the amount of O6-methyldeoxyguanosine present (11).
Limits of detection were 0.2 pmol/μmol of unmodified base. Quanti-
fication by fluorescence-linked HPLC or competitive radioimmunoassay
gave similar values for O6MG in respiratory or olfactory mucosa from
rats treated with 3 mg/kg NNK.

RESULTS

Dose Response for DNA Methylation by NNK in Respiratory
and Olfactory Mucosa. The dose response for O6MG and 7-
methylguanine in respiratory and olfactory mucosa was deter-
mined 4 h after treatment of rats (i.p. injection) with doses of
NNK ranging from 0.3 to 100 mg/kg. The molecular dosimetry
of O6MG formation differed considerably between respiratory
and olfactory mucosa. The relationship between dose and
O6MG formation was nonlinear in respiratory mucosa. The
dose-response curve was very steep from 0.3 to 3 mg/kg but
decayed markedly in the dose range of 10 to 100 mg/kg (Fig.
1A). In contrast, the dose response for the olfactory mucosa
did not demonstrate such a large change in slope over the same
dose range (Fig. 1B). The ratio of O6MG formed to dose of
NNK, an index of efficiency of alkylation, increased from 2.4
to 20 in respiratory mucosa as the dose of NNK was decreased
from 100 to 0.3 mg/kg (Fig. 2A). Alkylation efficiency in the
olfactory mucosa was not affected as the dose of NNK was
altered (Fig. 2B). A similar dose response for O6MG formation
was observed when NNK was administered via s.c. rather than
i.p. injection (data not shown).

Fig. 1. Dose response for DNA alkylation in nasal mucosa. O6-Methylguanine
and 7MG were determined in respiratory (A, •) and olfactory (B, O) mucosa
from rats 4 h after treatment with 0.3, 1.0, 3.0, 10, 30, and 100 mg/kg NNK.
The concentration of DNA adducts was determined as described in "Materials
and Methods." The measurement of 7MG in respiratory mucosa after treatment
with 0.3 mg/kg NNK and in olfactory mucosa following treatment with 0.3 or
1.0 mg/kg NNK was not possible because the concentration of this adduct was
below limits of detection by fluorescence-linked HPLC. Respiratory and olfactory
mucosa were pooled from two rats for measurement of DNA adducts. Points,
average of three to five separate determinations; values, means ± SEM.
Conditions were as described in Fig. 1. Concentrations of O'MG and 7MG were sacrificed 4 h after treatment on days 1, 2, 4, 6, and 12, and the concentration of DNA adducts determined as described in "Materials and Methods." Other conditions were as described in Fig. 1. Concentrations of O'MG and 7MG were significantly greater in respiratory than olfactory mucosa (P < 0.05) as determined by matched-paired Student's T test. Values, means ± SE from three to five pairs of rats.

Table 1 Formation of DNA adducts in nasal mucosa during treatment with NNK

Rats were treated for 12 consecutive days with NNK (10 mg/kg/day). Animals were sacrificed 4 h after treatment on days 1, 2, 4, 6, and 12, and the concentration of DNA adducts determined as described in "Materials and Methods." Other conditions were as described in Fig. 1. Concentrations of O'MG and 7MG were significantly greater in respiratory than olfactory mucosa (P < 0.05) as determined by matched-paired Student's T test. Values, means ± SE from three to five pairs of rats.

The concentration of 7MG was approximately 12-fold greater than O'MG in nasal mucosa after 1 dose of NNK (Table 1). Concentrations of 7MG were maximal in both regions after 2 days treatment and remained constant over the remaining 10 days of NNK administration. The ratio of O'MG to 7MG was similar in both regions of the nose and increased from 0.08 to 0.12 after 4 days of treatment with NNK. No further changes in this ratio were observed during the remaining 8 days of treatment. The fact that the O'MG/7MG ratio did not decrease during carcinogen treatment indicates that the removal of the O'MG adduct by O'MG-DNA methyltransferase was probably not induced in the nose.

Morphological Changes in the Nasal Passages during Treatment with NNK. Cytotoxicity in the respiratory and olfactory regions was determined following treatment of rats with 1, 10, 30, or 100 mg/kg NNK for up to 12 days. Histopathological examination of the nasal passages revealed both dose related and cell specific differences in toxicity as evidenced by necrosis or metaplasia. No toxicity was evident in the nose when 1 mg/kg NNK was administered (Table 2). The earliest morphological changes were observed after treatment for 2 days with 10 mg/kg NNK and involved the selective necrosis of the secretory cells of the lateral nasal glands (Steno's gland; Table 2) underlying the respiratory mucosa. Continued treatment and/or higher doses of NNK resulted in the loss of up to 95% of the acini and intercalated ducts of Steno's gland while striated ducts remained unaffected (compare Fig. 3, A, B, and C). Necrosis of Bowman's glands underlying the olfactory mucosa was evident after 2 days of treatment with 30 mg/kg NNK (Table 2; compare Fig. 3, D and E). Nuclear degeneration in the form of pyknosis and karyorrhexis as well as individual cell loss were greatest in the dorsomedial parts of the olfactory area (Fig. 3F ). Damage to both Steno's and Bowman's glands progressed in a dose and time dependent manner (Table 2). Serous glands underlying the respiratory mucosa were histologically normal during treatment with NNK.

Degeneration of the olfactory epithelium was observed after treatment for 4 days with 30 mg/kg NNK (Table 2; compare Fig. 3, G and H). Higher cumulative doses of carcinogen resulted in complete replacement of the olfactory epithelium by plump metaplastic cells (Fig. 3J). The respiratory mucosa was

Table 2 Dose response for toxicity in the nasal passages of the Fischer 344 rat during treatment with NNK

The dose response for toxicity in the nasal passages was determined in rats treated with 1, 10, 30, or 100 mg/kg NNK for 2, 4, or 12 days. Three to four rats were used for each dose and time point.

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<th>4 Days</th>
<th>12 Days</th>
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<tr>
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<td>100</td>
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<td></td>
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* Degree of necrosis or metaplasia graded from - to ++++, either severe loss of glands or total replacement of normal cells with metaplastic basal cells.
Fig. 3. Morphological changes in the nasal cavity during treatment with NNK. Rats were treated with 1, 10, 30, or 100 mg/kg NNK for up to 12 days. Animals were sacrificed after 2, 4, or 12 days of treatment and nasal passages were processed as described in "Materials and Methods." A–C, transverse sections just posterior to the upper incisor teeth; D–F, region of ethmoturbinates; G–I, dorsal nasal septum of the nasoturbinate region of control (A, D, and G) or treated rats (E, 4 days at 10 mg NNK/kg/day; B, 2 days at 30 mg NNK/kg/day; H, 12 days at 10 mg NNK/kg/day; C, F, and I, 12 days at 30 mg NNK/kg/day). a, architecture of the lateral nasal gland includes acini; ic, intercalated ducts; and sd, striated ducts. In B, selective necrosis of secretory cells is demonstrated by pyknotic (p) and karyorrhectic (k) nuclei; C, severe loss of acini and intercalated ducts, while striated ducts are unaffected. Architecture of the olfactory mucosa (D and G) includes sustentacular cell nuclei (sn), neural (sensory) cells (n), basal cells (b), olfactory nerve bundles (on), Bowman's glands (bg), and Bowman's gland ducts (d). Bowman's glands are prominent in the olfactory mucosa of D, reduced in size and number in E, and absent in F. Note the degeneration of olfactory epithelium (oe), as shown by pyknosis and loss of individual cells (H), and replacement of olfactory mucosa by large basal cells (I). [1-μm Glycol methacrylate embedded sections stained with toluidine blue. A, B, C, G, H, and I, × 212; D–F, × 320; B (insert), × 512].
relatively resistant to toxicity by NNK and exhibited only mild metaplasia after treatment for 12 days with 30 mg/kg while mild to moderate hyperplasia was evident following treatment for 12 days with 100 mg/kg (Table 2). Pathological changes were not detected in the epithelium of Jacobson's organ, the submucosal glands lining the ventral half of the septum, or in the group of glands associated with the dorsal, medial, and lateral parts of the maxillary sinus.

Localization of NNK in the Nasal Passages. The autoradiographic distribution of NNK within the nose was determined in rats exposed to 1 mg/kg [3H]NNK (specific activity 1.4 Ci/mmol). Four h after treatment, silver grains were more heavily concentrated over the lateral nasal glands (Steno's glands), Bowman's glands, and serous glands of the respiratory mucosa. The respiratory epithelium contained more silver grains per cell than the olfactory epithelium (Fig. 4).

Morphological Changes, Tumor Incidence, and Distribution in the Nasal Passages following 20 Weeks of Treatment with NNK. A carcinogenicity study was initiated to determine whether chronic treatment with NNK produces morphological changes and neoplasia which parallel the localization of acute cytotoxicity. Since significant toxicity to the olfactory region was observed after treatment with either 30 or 100 mg/kg, an

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Fig. 4. Microautoradiographic localization of NNK in the nasal cavity. Transverse sections through the region of the dorsal nasoturbinate of a rat 4 h after an i.p. injection of 1 mg/kg [3H]NNK (1.4 mCi/rat). Serous glands (sg) beneath respiratory epithelium (re) and Bowman's glands (bg) beneath the olfactory epithelium have the heaviest concentration of silver grains. The respiratory epithelium is more moderately labeled, and the olfactory epithelium (oe) is weakly labeled. Silver grains appear less numerous over goblet cells (gc) than ciliated cells due to apical vacuoles. (1 μm 50% glycol:50% methyl methacrylate embedded sections stained with toluidine blue, × 320).

Fig. 5. Histopathological changes in the nasal passages following treatment with NNK for 20 weeks. Rats were treated for 20 weeks with NNK (50 mg/kg, three times a week, s.c.) and sacrificed over a 35-week period after cessation of treatment. A, ethmoturbinite with hyperplasia and dilation of Bowman's glands (bg) and metaplasia of the olfactory epithelium (oe); B, ethmoturbinite has been thickened by an anaplastic carcinoma (arrow) believed to have arisen from Bowman's glands. There is metaplasia of the intact olfactory epithelial surface (oe); C, a polypoid adenoma (arrow) protrudes from the mucosal surface in the respiratory region of the nasal cavity. (H & E: A, × 75; B, × 150; C, × 100).
intermediate dose of 50 mg/kg was chosen for the carcinogenicity study. Based on the dose response for O6MG formation (Fig. 1), this concentration of NNK should result in similar alkylation levels in both regions of the nose. Male Fischer 344 rats were treated 3 times a week for 20 weeks with either NNK (50 mg/kg, s.c.) or vehicle (trioctanoin). This treatment regimen was chosen because it has previously been shown to induce nasal tumors (2). One interim sacrifice was performed where six treated and three control rats were killed 9 weeks after treatment was discontinued. The remaining animals were sacrificed 3 times a week for 20 weeks while 17 rats were treated with vehicle (trioctanoin) following the same dosing regimen. Animals in the treatment group were killed after a 10–15% loss of body weight over the course of 35 weeks following cessation of treatment. Nasal passages were removed, fixed in neutral buffered formalin, and processed for histological examination as described in "Materials and Methods."

Table 3 Tumor incidence and distribution in the nasal passages of the Fischer 344 rat following chronic treatment with NNK

<table>
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<th>Location of lesion</th>
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<th>Malignant</th>
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<td>Respiratory region</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steno's gland</td>
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<tr>
<td>Serous gland</td>
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<td>Respiratory epithelium</td>
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<tr>
<td>Bowman's gland</td>
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with NNK were observed in the nasal passages of the rat. The high levels of DNA alkylation in the nose parallel earlier metabolism and autoradiographic studies (13, 14). Cultured rat nasal mucosa metabolize NNK to hydroxylated products (13) and autoradiographic studies with [carbonyl-14C]NNK have shown a strong binding of radioactivity to nasal tissue (14). Metabolism studies by Hecht et al. (3) have suggested that in addition to methylation of DNA, NNK could undergo hydroxylation at the N-methyl carbon and subsequent hydrolysis to yield a pyridyloxobutyl diazohydroxide. This intermediate could then react with DNA to form a bulky adduct. Small amounts (approximately 0.1 pmol/μmol deoxyguanosine) of this adduct have been identified in liver after multiple administrations of [5-3H]NNK to rats (15). Recent studies have demonstrated a slight increase in the incidence of nasal tumors following α-deuterium substitution of NNK (16). However, at present there is no data to confirm whether a bulky adduct formed from NNK is responsible for this increase in neoplasia. There is also no information on the removal of this adduct or its ability to initiate base mispairing. In contrast, methylation of DNA by NNK has been studied extensively in several tissues (4, 17) and cell types (18).

The quantitation of O6MG in hepatic and extrahepatic tissues from rats during treatment for 12 days with NNK (100 mg/kg/day) indicated that the concentration of this adduct was three to four times greater in nasal mucosa than in liver or lung over the same treatment period (4). The concentration of O6MG was similar in respiratory and olfactory mucosa after treatment with high doses of NNK (30 or 100 mg/kg). However, the present study indicates that the molecular dosimetry of O6MG in DNA of respiratory and olfactory mucosa differ markedly. The concentration of O6MG was two to four times greater in respiratory than olfactory mucosa after treatment with doses of NNK ranging from 0.3 to 3.0 mg/kg (Fig. 1) and 60 to 90% higher during multiple dose administration (10 mg/kg/day). Differences in alkylation in the two regions of the nose probably cannot be attributed to substrate delivery since the nasal epithelium in both regions of the nose has a rich blood supply (18) and NNK was administered via i.p. injection. It is also unlikely that differences in the rate of removal of O6MG influence the distribution of this adduct in the nose because O6MG-DNA methyltransferase activity was only slightly greater (25%) in the respiratory region of the nose. The difference in the shape of the dose-response curves for O6MG in respiratory and olfactory mucosa may stem from the localization of metabolic pathways for biotransformation of NNK. The amount of O6MG formed per unit dose of NNK increased 8-fold in respiratory mucosa as the dose of carcinogen was decreased from 100 to 0.3 mg/kg (Fig. 2). A similar alkylation efficiency curve for O6MG has also been observed in rat lung after treatment with NNK (19).

Based on the two component nature of this curve, we hypothesized previously (19) that low and high Km pathways exist for activation of NNK to a methylating agent in the lung. The fact that the concentration of O6MG was greater in respiratory than olfactory mucosa and that the efficiency for alkylation increased in respiratory mucosa but remained constant in the olfactory mucosa after low dose exposure suggests that a low Km pathway for NNK activation is also present in the nose and that this pathway is localized predominantly in the respiratory region. This conclusion is supported by autoradiographic studies which

3 S. A. Belinsky, unpublished observation.
demonstrated that silver grains were more heavily concentrated over respiratory than olfactory epithelium (Fig. 4) 4 h after treatment of rats with 1 mg/kg NNK.

Since DNA isolated from respiratory mucosa is selectively alkylated after low doses of NNK, one would hypothesize that this region would also exhibit greater sensitivity for cytotoxicity and neoplasia than the olfactory mucosa following carcinogen exposure. However, no toxicity was observed in either portion of the nasal passages (Table 2) using the dosing regimen which gave the largest differences in alkylation between the two regions of the nose (1 mg/kg). Following exposure to higher doses of NNK, the respiratory mucosa and underlying glands were more resistant to toxicity and neoplasia exhibiting only mild metaplasia and a 5% incidence of malignant tumors. In contrast, treatment with NNK resulted in marked necrosis of the Bowman's glands and olfactory epithelium leading initially to hyperplasia and basal cell metaplasia and finally to a 45% incidence of malignant tumors in this area of the nose. Furthermore, more animals probably would have developed nasal tumors had there not been competing causes for morbidity (liver or lung tumors). Based on autoradiography and alkylation studies, it is apparent that the differential toxicity to the nasal passages induced by NNK cannot be attributed to either the local concentration of DNA adducts or to the NNK itself. At present, the mechanism for this selective toxicity is not understood.

The spectrum of proliferative changes in Bowman's glands included hyperplasia, benign neoplasia, and malignant neoplasia. Clear evidence of Bowman's gland hyperplasia was found in all NNK-treated rats including those with malignant neoplasms. In several rats, benign or malignant neoplasms could be observed arising within areas of Bowman's gland hyperplasia. Because of the ubiquity of this spectrum of proliferative changes in Bowman's glands, it is presumed that the large carcinomas that had destroyed normal turbinate structures in the olfactory region also arose from Bowman's glands. Similar nasal histopathological changes were observed in rats held for up to 60 weeks following treatment (i.p. administration) with 12 daily doses of 100 mg NNK per kg body weight. It is apparent from these studies that the route of administration (s.c. for the 20-week exposure, and i.p. for the 12-day exposure) does not alter the spectrum of morphological changes observed in the nose. Furthermore, treatment for 12 days with a dose of NNK (100 mg/kg/day) which causes significant toxicity to the olfactory region appears sufficient to cause cellular transformation and progression to neoplasia. The data in this study also indicate that the majority of tumors induced by NNK in the nasal passages probably originate from the Bowman's glands.

One of the major shortcomings in estimating carcinogenic risk from long term exposure to chemical carcinogens is the uncertainty of extrapolating from high to low doses. Recent studies (20) have suggested that a more accurate estimation of risk would relate tumor response to the target organ concentration of DNA adducts rather than to the applied dose. However, these experiments indicate that adduct concentration alone may not be a good index of carcinogenic risk in the nose from NNK. Based solely on the alkylation efficiency curves for O^6MG (Fig. 2), one would conclude that the probability for initiation of tumorigenesis should be much greater in respiratory than olfactory regions after low dose exposure to NNK. However, the majority of malignant tumors induced by NNK appear to originate from the olfactory region and occur only after chronic treatment with high doses of NNK (21; Table 3). The steep dose-response curve for induction of tumors (21) as well as the localization of lesions in the nasal passages can be explained by a difference in sensitivity to the cytotoxicity of NNK. Although the concentration of O^6MG was greater in respiratory regions after low dose exposure to NNK, no cytotoxicity was observed in either region of the nose. In contrast, after exposure to high doses of the carcinogen, adduct concentrations were similar in both regions of the nose, however marked cytotoxicity was localized to the olfactory region. Cell proliferation in the control Fischer 344 rat is very low (0.1 to 0.2%) in both respiratory and olfactory epithelium (22). However, a marked increased in cell proliferation in the olfactory region demonstrated by severe basal cell metaplasia secondary to NNK-induced necrosis could result in mutation at the site of DNA adducts, clonal expansion of initiated cells, and a much greater probability of cells undergoing subsequent "critical" mutations. Taken together, these data indicate that both the formation of promutagenic adducts and cell proliferation secondary to toxicity are required for the induction of neoplasia by NNK within the nose.

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DNA ALKYLATION AND CELL TOXICITY IN NASAL MUCOSA


Molecular Dosimetry of DNA Adduct Formation and Cell Toxicity in Rat Nasal Mucosa following Exposure to the Tobacco Specific Nitrosamine 4-(N-Methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone and Their Relationship to Induction of Neoplasia

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