Carcinogenicity of Nickel Subsulfide for Respiratory Tract Mucosa¹

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ABSTRACT

The carcinogenicity of nickel subsulfide, Ni₃S₂, for respiratory tract epithelium was studied in heterotopic tracheal transplants with doses of 1 and 3 mg Ni₃S₂ per trachea. Chemical determinations indicated that Ni₃S₂ persisted in the tracheas for seven to nine months. Ni₃S₂ showed marked toxicity for mucociliary epithelium, resulting in widespread atrophy and focal epithelial necrosis during the first two months of exposure. The submucosa showed mononuclear infiltration and signs of fibroblastic and capillary proliferation.

Tumor studies indicated that Ni₃S₂ can induce carcinomas in tracheal epithelium. The carcinoma incidence was 10% at 1 mg and approximately 1.5% at 3 mg. The higher dose produced a 67% incidence of fibro- and myosarcomas. The data suggest that, compared to some carcinogenic polycyclic hydrocarbons, Ni₃S₂ may not be a strong carcinogen for the epithelium of conducting airways. The data are discussed in light of other experimental studies and of epidemiological findings on respiratory tract cancers in nickel workers.

INTRODUCTION

A number of epidemiological studies have shown that nickel workers have a markedly elevated risk of developing respiratory tract cancers (3, 15, 16, 21, 22). Metallic nickel subsulfide, Ni₃S₂, and nickel oxide are suspected of being the major air contaminants responsible for this high cancer incidence. Experimental studies have confirmed the epidemiological findings and shown that sarcomas can be induced readily by s.c. or i.m. injections of various nickel compounds (6). (For review of nickel carcinogenesis see Refs. 23 and 24.) However, relatively few attempts have been made to investigate the susceptibility of the respiratory tract to nickel. Hueper (8) reported a small number of pulmonary tumors in guinea pigs exposed to nickel carbonyl, and Ottolenghi et al. (17) reported induction of pulmonary adenomas and adenocarcinomas in rats after chronic inhalation of Ni₃S₂ dust. The tumor induction time in all of these studies was close to 2 years. Compared to tumor latencies observed following s.c. (7), i.m. (4, 27), i.r. (9), or i.t. (2) injections of nickel, this seems to be a surprisingly long time (and the tumor incidence seems to be low), suggesting that the respiratory tract might be more resistant to nickel carcinogenicity. However, such comparisons are difficult to make since no data are available that allow comparison of nickel concentration as a function of time in the respective tissues. That the concentrations of nickel in the lungs following inhalation are considerably below that achieved at various injection sites (e.g., kidney or muscle) is not unlikely.

Because of the sparsity of information on the sensitivity of respiratory tract mucosa to pure nickel compounds, we decided to investigate the problem in a system that allows the test compound to stay in intimate contact with the respiratory mucosa for protracted periods of time. The tracheal transplant system (5, 11) seemed to be well suited for this purpose. The test substance is introduced into the lumen of established subcutaneous tracheal grafts. Exposure continues as long as the test substance remains in the lumen, i.e., weeks or months, depending on the solubility of the material or the release rate from the vehicle in which it was delivered (18).

By using this system we found that, at starting doses of 3 mg Ni₃S₂ per trachea, a high incidence of fibro- and leiomyosarcomas occurred with a relatively short induction time. Only 1 of 64 tracheas developed a carcinoma. At 1 mg Ni₃S₂ per trachea, low incidences of carcinomas (10%) and sarcomas (5%) occurred with tumor induction times of more than one year. The significance of our findings is discussed.

MATERIALS AND METHODS

The animals used in this study were 10-week-old female Fischer 344 rats. Tracheas were obtained from donor animals, were tied to polyethylene tubing, and transplanted under the dorsal skin of isogenic recipients 2 tracheas per rat (5, 11). In all, 348 grafted tracheas were used in this study. Ni₃S₂ (purity, 98 to 99%; particle size, 2.8 μm) was generously supplied by Dr. F. W. Sunderman, Jr. (Laboratory of Medicine, University of Connecticut School of Medicine, Farmington, Conn.), and by the International Nickel Company of Canada (J. Roy Gordon Research Laboratory, Sheridan Park, Mississauga, Ontario, Canada). Gelatin pellets were prepared with either 1 or 3 mg Ni₃S₂; control pellets contained 20% gelatin without Ni₃S₂.

Four weeks after grafting, when the tracheas were well vascularized and fully established with a normal mucosa (5), pellets containing the test substance or control pellets were inserted into the grafts through a small incision at the bifurcational end. Nickel was determined by IDSSMS. Many sample types and elements have been determined by

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RESULTS

NiS2 Determinations. The amounts of NiS2 detected in tracheas at different times after the start of exposure are summarized in Table 1. The rate of disappearance of NiS2 was similar in the 2 groups: approximately 25% had disap-

<table>
<thead>
<tr>
<th>NiS2 (mg) remaining from pellets originally containing</th>
<th>Time (mos.)</th>
<th>1 mg</th>
<th>3 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.745 ± 0.02</td>
<td>2.26 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.49 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.132 ± 0.038</td>
<td>0.515 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.115 ± 0.02</td>
<td>0.086 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.055 ± 0.007</td>
<td>0.013 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.019 ± 0.011</td>
<td>0.007 ± 0.002</td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± SD.

The submucosa showed diffuse lymphocytic infiltration. At 1 and 2 months, atrophy and flattening of the epithelium was the predominant change. After 3 months most of the epithelium was indistinguishable from that observed in controls, except for a few small patches of atrophic and thin squamous epithelium. More conspicuous than the epithelial reactions was the mononuclear cell infiltrate in the submucosa.

With 3 mg of NiS2, the most common early epithelial reaction was "transitional hyperplasia," i.e., multilayered undifferentiated epithelium made up of large polyhedral cells. In addition, focal squamous metaplasia and patches of atrophic epithelium were observed. At 2 to 4 weeks the toxic changes became even more dramatic; "bizarre" cells, i.e., very large irregularly shaped cells with giant nuclei, were common and focal epithelial necrosis occurred. Particles, assumed to be NiS2, were found in the connective tissue, particularly in the areas of epithelial erosion. The submucosa showed a general increase in cellular elements and a heavy lymphocytic infiltration. At 2 to 4 months, atrophic epithelial changes were predominant. Hyperplastic and metaplastic foci were also seen. The submucosa showed an increase in fibroblasts, capillaries, and what appeared to be myoblasts. After 5 months the epithelial morphology no longer changed very much; atrophic and flattened epithelium was most widespread, interspersed with hyperplastic and metaplastic patches. The alterations of the submucosa were much more conspicuous; tissue mass was dramatically increasing, leading to a constriction of the tracheal lumen and, at 7 to 9 months, complete obstruction of the lumen. At 9 months the first sarcomas were diagnosed. The sequential morphological changes are illustrated in Fig. 1.
were sarcomas and 6 (10%) were carcinomas (2 squamous
were located outside the trachea and were probably due to
which were attributed to NiS2 exposure (15%). The other 3
tumors was a carcinoma (of the squamous type; 1.5%). All
sion into host tissues but no distant métastases.
13 and 15 months, respectively, showed extensive inva
the polyethylene tubing. Of the 9 trachéal tumors, 3 (5%)
comas, 1 as a leiomyosarcoma. The 3 carcinomas detected
Of the 3 trachéal sarcomas, 2 were diagnosed as fibrosar-
mas, 1 as a leiomyosarcoma. The 3 carcinomas detected
at 13 and 15 months, respectively, showed extensive inva-
no pellets, and had been maintained for 20 or more months, the sarcoma inci-
dence was 11%. All of these tumors developed outside the trachea
around the polyethylene tubing, with the first sarcoma
remaining at 1 month and roughly 15% at 3 months. The 2
NiS2 doses applied in our experiments are within the same
range chosen by other investigators for i.m. (25, 26) and i.r.
injection studies (9). The sarcoma incidence reported by
Sunderman et al. (25), following i.m. inoculations of NiS2
into Fischer 344 rats, was 23, 77, and 93% for doses of 0.6,
1.2, and 2.5 mg, respectively, of NiS2. In our experiments
the nickel-related tumor incidence was 15 and 69% at doses
of 1 and 3 mg, respectively. The tumor incidence reported
by Jasmin and Riopelle (9) following i.r. injection of 5 mg
NiS2 was approximately 45% (within 12 months). Thus it
seems that the sensitivity of the tracheal tissues to the
carcinogenicity of NiS2 is roughly comparable to that of
other tissues, although minor differences may exist. How-
ever, it must be remembered that the vast majority of the
tumors observed in our experiments were sarcomas, and
only 5% of the tracheas in the 2 dose groups combined
(namely, 7 of 124 tracheas) developed carcinomas. This 5%
carcinoma incidence is surprisingly low for a carcinogen
that is highly suspected of being the major etiological agent
in respiratory cancer development of occupational groups
exposed to nickel compounds (for review and literature see
Refs. 23 and 24). A partial explanation for this unexpected
finding may be provided by our own observations. The
carcinoma incidence in the 3-mg NiS2 group is artificially
low because of the high and early sarcoma incidence. Thus
many tracheas were not at risk to develop carcinomas after
1 year. In addition the serial histopathological studies
suggest that NiS2 is highly toxic for trachéal epithelium, as
manifested by widespread atrophy and, particularly at the
higher NiS2 dose, epithelial necrosis. Thus the toxicity can
easily outweigh the carcinogenicity and result in a mislead-
ningly low carcinoma incidence unless a very detailed dose-
response study is carried out. However, with only one
exception, namely, the carcinoma occurring in the 3-mg
group, the tumor induction time for all carcinomas in this
study was 13 months or more. Three early invasive carci-
nomas were discovered histologically at the end of the
study (20 months). Thus the possibility cannot be ruled out
at this time that tracheal epithelium may be relatively
resistant to nickel carcinogenicity. This lends support to
the idea that other factors might also be involved in the
causation of respiratory tract cancers in nickel workers
(23). Several experimental studies (10, 14, 28) provide direct

**DISCUSSION**

Our data show that, in the tracheal transplant system, it is
possible with a single inoculation of NiS2 crystals to
achieve an exposure of respiratory mucosa lasting 7 to 9
months. The half-life of nickel in trachéal transplants ap-
pears to be not too dissimilar from that observed in i.m.
injection studies (25). Approximately 50% of the nickel
remained at 1 month and roughly 15% at 3 months. The 2
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range chosen by other investigators for i.m. (25, 26) and i.r.
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**Table 2**

**Tumor induction study with NiS2**

<table>
<thead>
<tr>
<th>NiS2 pellet (mg)</th>
<th>No. of effective tracheas</th>
<th>No. of tracheas with sarcoma</th>
<th>No. of tracheas with carcinoma</th>
<th>No. of tracheas with tumors at various times after start of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>6</td>
<td>6</td>
<td>1S (2) 2C 1C 1S 1S 3S c 3C (7)</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>44</td>
<td>1</td>
<td>2S 14S 4S 9S e 2S 5S 5S 1S 1S 1S</td>
</tr>
</tbody>
</table>

a At 20 months all surviving animals were killed.
b S, sarcoma; C, carcinoma.
c These 3 sarcomas occurred extratracheal and are considered not to be NiS2 related.
d Number in parentheses, number of tracheas examined at that time interval.
e Includes 1 tumor diagnosed as benign myoma.

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evidence that polycyclic hydrocarbons and nickel compounds administered simultaneously result in an enhanced tumor response compared to that of each chemical alone.

REFERENCES


Fig. 1. Sequential changes in transplanted tracheas, induced by Ni₅S₄. a: mild columnar hypertrophy of tracheal epithelium at 1 week, induced by 1 mg Ni₅S₄, H & E, × 300. b: epithelial atrophy at 1 month, induced by 1 mg Ni₅S₄; subsequently the epithelium became indistinguishable from that in controls. H & E, × 300. c: transitional hyperplasia at 1 week, induced by 3 mg Ni₅S₄, H & E, × 300. d: squamous metaplasia at 1 week, induced by 3 mg Ni₅S₄, H & E, × 300. e: epithelial necrosis induced by 3 mg Ni₅S₄, at 1 month. Note Ni₅S₄ crystals in tracheal lumen. H & E, × 300. f: retention of Ni₅S₄ in submucosal tissue after exposure to 3 mg Ni₅S₄ for 2 months. Note thickening of submucosa. H & E, × 190. g: occlusion of tracheal lumen by connective tissue following exposure to 3 mg Ni₅S₄, at 7 months. H & E, × 30. h: development of sarcoma (†) in connective tissue, occluding tracheal lumen, at 10 months of exposure to 3 mg Ni₅S₄. H & E, × 30. Right, early sarcoma. × 250.
Fig. 2. Tracheal tumors induced by Ni₃S₂. a, early invasive carcinoma. H & E, x 30. b, squamous cell carcinoma. c, undifferentiated carcinoma. d, fibrosarcoma. e, rhabdomyosarcoma. f, undifferentiated sarcoma. b to f: H & E, x 300.
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