Carcinogenicity of Nickel Subsulfide for Respiratory Tract Mucosa

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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 dust by inhalation. Sunderman and Donnelly (20) found a low incidence of pulmonary carcinomas in rats after inhalation of 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 isogeneic 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 isotope dilution spark source mass spectrometry.

* The abbreviations used are: i.r., intrarenal; i.t., intratesticular; IDSSMS, isotope dilution spark source mass spectrometry.
IDSSMS (1, 12, 19). The trachea samples were wet ashed, and the resulting solution was spiked with known quantities of $^{61}$Ni (1.62% $^{59}$Ni, 5.18% $^{60}$Ni, 92.11% $^{61}$Ni, 1.04% $^{62}$Ni, and <0.05% $^{63}$Ni). The equilibrated solutions were dried on graphite electrodes and sparked in the ion source of the mass spectrometer (AEI MS-7). The ions produced were recorded photographically, and the ion intensities of the lines on the photographic plate were determined by a densitometer data system (13). Concentration of nickel was determined from the ratio of $^{61}$Ni to $^{58}$Ni in the spiked sample.

Nickel recovery was determined by the addition of known amounts of Ni$_2$S$_2$ to a homogenized liver sample. Recovery was always >95%, and the sensitivity of IDSSMS was sufficient to measure about 1 µg nickel per g tissue. Reproducibility of the method was about 5% of the value when the concentrations were >10 µg nickel per g tissue.

The alternate method for the determination of nickel in the trachea samples was by direct-reading optical emission spectrometry with radio frequency spark excitation of the sample from a rotating disc electrode. Concentrations of nickel were determined from a calibration curve obtained by plotting the ratio of nickel at 3101.55 Å to strontium at 40.77.1 Å against nickel concentration in standard solutions containing strontium as an internal standard. Background at 4401 Å was electronically compensated, and blanks were determined for each series of analyses. Reproducibility was about 5% of the value at nickel concentrations >100 µg/g tissue. (The determinations were carried out by J. C. Franklin, Analytical Chemistry Division, Oak Ridge National Laboratory.)

At different times after insertion of nickel or control pellets, 2 to 3 tracheas were removed from host animals and processed for histology to determine the pathological changes developing as a function of exposure duration. Tracheas were sampled at 0.25, 0.50, 1, 2, 3, 4, 5, 7, 9, and 12 months. Tracheas were cut transversely into 10 to 14 rings, fixed in Bouin's fixative, and embedded in paraffin, maintaining the proper sequence of rings.

For the tumor induction study, 60 tracheas received pellets containing 1 mg, and 64 tracheas received pellets containing 3 mg Ni$_2$S$_2$; 10 tracheas received control pellets over 200 historical controls exist in our laboratory from various studies). The tracheas were palpated twice a month to determine the time of tumor development. When progressively enlarging tumors reached 2.0 cm in diameter, they were surgically removed, together with the tracheas, and processed for histology. The other trachea on the same recipient remained in place until tumor development or the end of the study. At 20 months the experiment was terminated and the remaining tracheas were processed for histology. Tissues were stained with hematoxylin and eosin and with special connective tissue stains when indicated. Complete necropsies were carried out on all animals.

**RESULTS**

**Ni$_2$S$_2$ Determinations.** The amounts of Ni$_2$S$_2$ detected in tracheas at different times after the start of exposure are summarized in Table 1. The rate of disappearance of Ni$_2$S$_2$ was similar in the 2 groups: approximately 25% had disappeared at 1 week, 50% disappeared at 1 month, and 85% disappeared at 3 months. By 5 months the amount of Ni$_2$S$_2$ recoverable from tracheas of either group had dropped to approximately 0.1 mg/trachea and continued to decline thereafter but not in a fashion related to the starting dose.

**Table 1**

<table>
<thead>
<tr>
<th>Time (mos.)</th>
<th>1 mg</th>
<th>3 mg</th>
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<tbody>
<tr>
<td>0.25</td>
<td>0.745 ± 0.02$^a$</td>
<td>2.26 ± 0.22</td>
</tr>
<tr>
<td>1</td>
<td>1.49 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.132 ± 0.038</td>
<td>0.515 ± 0.17</td>
</tr>
<tr>
<td>5</td>
<td>0.115 ± 0.02</td>
<td>0.086 ± 0.005</td>
</tr>
</tbody>
</table>
| 7          | 0.055 ± 0.007  | 0.013 ± 0.0 |\[
| 9          | 0.019 ± 0.011  | 0.007 ± 0.002 |

$^a$ Mean ± SD.

**Sequential Histological Abnormalities.** Morphological changes in the tracheas receiving control pellets were transient, showing mild epithelial hyperplasia during the first 2 weeks.

With 1 mg of Ni$_2$S$_2$, the epithelial changes observed during the first 2 weeks were minor. The most conspicuous finding was a generalized hypertrophy with tall columnar cells; hyperplasia occurred only in isolated small areas. 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 Ni$_2$S$_2$, 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 Ni$_2$S$_2$, 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, to complete obstruction of the lumen. At 9 months the first sarcomas were diagnosed. The sequential morphological changes are illustrated in Fig. 1.

**Tumor Induction.** The 10 control tracheas were examined...
histologically at 20 months. No tumors were observed. The tracheas were lined by low cuboidal mucociliary epithelium and were filled with mucus. In more than 200 historical controls of trachea transplants, which had received either gelatin pellets, beeswax pellets, or no pellets, and had been maintained for 20 or more months, the sarcoma incidence was 11%. All of these tumors developed outside the trachea around the polyethylene tubing, with the first sarcoma appearing at 20 months. In contrast the mean induction time of the sarcomas believed to have developed as a result of NiS₂ exposure (15%). The other 3 tumors were located outside the trachea and were probably due to the polyethylene tubing. Of the 9 tracheal tumors, 3 (5%) were sarcomas and 6 (10%) were carcinomas (2 squamous cell carcinomas at 13 months, 1 undifferentiated carcinoma at 15 months, 3 squamous cell carcinomas at 20 months). Of the 3 tracheal sarcomas, 2 were diagnosed as fibrosarcomas, 1 as a leiomyosarcoma. The 3 carcinomas detected at 13 and 15 months, respectively, showed extensive invasion into host tissues but no distant metastases.

With 3 mg of NiS₂, the first tumor was observed at 6 months. Forty-five of the 64 tracheas developed tumors attributable to nickel exposure (70%). Only 1 of these tumors was a carcinoma (of the squamous type; 1.5%). All of the other tumors were diagnosed as sarcomas of various types (12 fibrosarcomas, 10 leiomyosarcomas, 10 fibromyxosarcomas, 2 rhabdomyosarcomas, 2 fibromyxosarcomas, 7 sarcomas of uncertain type, and 1 benign myoma). The tumors are illustrated in Fig. 2.

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 tracheal transplants appears 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 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. However, 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 tracheal 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 misleadingly 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 carcinomas 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

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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>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of tracheas with tumors at various times after start of exposure</th>
</tr>
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<tbody>
<tr>
<td>No. of tracheas with tumors at various times after start of exposure</td>
</tr>
<tr>
<td>6 mos.</td>
</tr>
<tr>
<td>1S b</td>
</tr>
<tr>
<td>2S</td>
</tr>
<tr>
<td>2S</td>
</tr>
</tbody>
</table>

* 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 Ni$_{32}$S$_2$. 

a: mild columnar hypertrophy of tracheal epithelium at 1 week, induced by 1 mg Ni$_{32}$S$_2$. H & E, x 300.

b: epithelial atrophy at 1 month, induced by 1 mg Ni$_{32}$S$_2$. Subsequently the epithelium became indistinguishable from that in controls. H & E, x 300.

c: transitional hyperplasia at 1 week, induced by 3 mg Ni$_{32}$S$_2$. H & E, x 300.

d: squamous metaplasia at 1 week, induced by 3 mg Ni$_{32}$S$_2$. H & E, x 300.

e: epithelial necrosis induced by 3 mg Ni$_{32}$S$_2$, at 1 month. Note Ni$_{32}$S$_2$ crystals in tracheal lumen. H & E, x 300.

f: retention of Ni$_{32}$S$_2$ in submucosal tissue after exposure to 3 mg Ni$_{32}$S$_2$ for 2 month. Note thickening of submucosa. H & E, x 190.

g: occlusion of tracheal lumen by connective tissue following exposure to 3 mg Ni$_{32}$S$_2$ at 7 months. H & E, x 30.

h: development of sarcoma (†) in connective tissue, occluding tracheal lumen, at 10 months of exposure to 3 mg Ni$_{32}$S$_2$. H & E, x 30. Right, early sarcoma. x 250.
Fig. 2. Tracheal tumors induced by NiS₂. a, early invasive carcinoma. H & E, × 30. b, squamous cell carcinoma. c, undifferentiated carcinoma. d, fibrosarcoma. e, rhabdomyosarcoma. f, undifferentiated sarcoma. b to f: H & E, × 300.
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