The Carcinogenicity of Intravenous Nickel Carbonyl in Rats

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SUMMARY

The carcinogenicity of i.v. nickel carbonyl, Ni(CO)₄, was tested in Sprague-Dawley rats. In 72 rats (Group A) that survived a single 50% lethal dose injection of Ni(CO)₄ (2.2 mg nickel per 100 g), the incidence of malignant tumors (8.3%) was not significantly greater than the incidence in 47 rats in the control group (4.3%). In 121 rats (Group B) that survived six 5% lethal dose injections of Ni(CO)₄ (0.9 mg nickel per 100 g) at intervals of 2 or 4 weeks, the incidence of malignant tumors (15.7%) was significantly greater than in the controls (p < 0.05). The 19 malignant tumors in Group B included 6 undifferentiated sarcomas (lung, pleura, liver, pancreas, uterus, and abdominal wall), 3 fibrosarcomas (neck, pinna, and orbit), 3 carcinomas (liver, kidney, and breast), 1 s.c. hemangioendothelioma, 1 leukemia, and 5 lymphomas (lung). This study demonstrates that multiple parenteral injections of Ni(CO)₄ can induce diverse malignant tumors in various organs and tissues of the rat.

INTRODUCTION

Nickel carbonyl is a toxic, volatile liquid which is an intermediate product in the Mond process for refining nickel matte and is used as a catalyst in the petroleum, plastic, and rubber industries. Nickel carbonyl has been implicated as a possible respiratory carcinogen in industrial atmospheres (4, 6) and in tobacco smoke (18). In rats that were exposed to inhalation of gaseous nickel carbonyl, Sunderman et al. (16, 17) found increased incidence of pulmonary carcinomas, and Sanina (15) observed increased incidence of malignant tumors of the uterus, ovaries, and mammary glands. The present study was performed in order to ascertain whether or not i.v. administration of liquid nickel carbonyl to rats results in increased incidence of malignant tumors. This investigation was stimulated by our findings that the acute pathological (9) and biochemical (12, 19, 26, 28) reactions that develop in rats after i.v. injections of nickel carbonyl are similar to those that occur following exposures to nickel carbonyl by inhalation.

RESULTS

As indicated in Table 1, the longevity of rats in the control group and in the 2 treated groups did not differ significantly. There were no significant differences between the incidences of benign tumors in the control group and in the 2 treated groups. In female rats, benign fibroadenomas of the breast were found in 13/46 (28%) of Group A, in 11/60 (18%) of Group B, and in 10/32 (31%) of the control group. In male rats, the only benign tumors were 2 small s.c. plexiform neuromas that were found in 2 rats of the control group. In the control group, the overall incidence of malignant tumors was 4.3%, which is consistent with published statistics for spontaneous malignant tumors in Sprague-Dawley rats (5, 29). There were no significant differences between the incidences of malignant tumors of male and female rats within the control group or within either of the treated groups. In rats of Group A that survived a single LD₅₀ injection of nickel carbonyl, the overall incidence of malignant tumors was 8.3%, which was not significantly greater than that in the control group treated with a single injection of nickel carbonyl.

MATERIALS AND METHODS

The experimental animals were randomly bred albino rats of the Sprague-Dawley strain (Dublin Farms, Dublin, Ga.), maintained on Purina laboratory rat chow. The rats were 8 to 10 weeks old at the time of the initial injection. Ni(CO)₄ (Matheson Chemical Co., East Rutherford, N.J.) was administered by i.v. injection into a tail vein by means of a microsyringe, as previously described (10, 11). The purity of the Ni(CO)₄ was verified by gas chromatography on 3 different chromatographic columns, as described by Sunderman et al. (27). Group A consisted of 72 rats (26 males, 46 females) that survived a single injection of Ni(CO)₄ in LD₅₀ dosage [2.2 mg nickel per 100 g body weight, equivalent to 5 μl of Ni(CO)₄ per 100 g body weight]. Group B consisted of 121 rats (61 males, 60 females) that survived 6 injections of Ni(CO)₄ in LD₅₀ dosage [0.9 mg nickel per 100 g body weight, equivalent to 2 μl of Ni(CO)₄ per 100 g body weight] administered at intervals of 2 or 4 weeks. The control group consisted of 47 rats (15 males, 32 females) that received sham injections of 5 μl of 0.9% (w/v) NaCl solution. The rats either died spontaneously or were killed when they became so moribund that they could not move about in their cages and hence could not obtain food or water. The rats were autopsied and the tissues were examined by light microscopy.
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Table 1

Incidence of malignant tumors in rats after i.v. nickel carbonyl

Sprague-Dawley rats in Group A survived 1 i.v. injection of Ni(CO)₄ at LD₅₀ dosage (2.2 mg nickel per 100 g). Rats in Group B survived 6 i.v. injections of Ni(CO)₄ at LD₅ dosage (0.9 mg nickel per 100 g) at intervals of 2 or 4 weeks. Rats in the control group received sham injections of 5 μl of 0.9% (w/v) NaCl solution.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of rats in group</th>
<th>Median age at death</th>
<th>Total incidence of malignant tumors</th>
<th>Pulmonary lymphomas</th>
<th>All other malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 (M)</td>
<td>22 (9–25)</td>
<td>3</td>
<td>11.5</td>
<td>2</td>
<td>7.7</td>
</tr>
<tr>
<td>46 (F)</td>
<td>23 (6–37)</td>
<td>6</td>
<td>6.5</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>72 (M, F)</td>
<td>23 (6–37)</td>
<td>8</td>
<td>8.3</td>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61 (M)</td>
<td>21 (5–30)</td>
<td>10</td>
<td>16.4</td>
<td>3</td>
<td>4.9</td>
</tr>
<tr>
<td>60 (F)</td>
<td>24 (8–36)</td>
<td>9</td>
<td>15.0</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>121 (M, F)</td>
<td>23 (5–36)</td>
<td>19</td>
<td>15.7</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (M)</td>
<td>24 (10–30)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>32 (F)</td>
<td>24 (6–33)</td>
<td>2</td>
<td>6.3</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>47 (M, F)</td>
<td>24 (6–33)</td>
<td>2</td>
<td>4.3</td>
<td>2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

- Numbers in parentheses, range.
- Fibrosarcoma (orbit).
- Cholangiocarcinoma (liver).
- Fibrosarcoma (orbit, pinna, neck); undifferentiated sarcoma (lung); hemangioendothelioma (s.c. tissue); undifferentiated leukemia; carcinoma (kidney).
- Undifferentiated sarcomas (pleura, liver, pancreas, uterus, abdominal wall); carcinomas (liver, breast).
- p < 0.05 versus control group ($\chi^2$ test).
- p < 0.02 versus control group ($\chi^2$ test).

In rats of Group B that received 6 injections of nickel carbonyl in LD₅ dosage, the overall incidence of malignant tumors was 15.7%, which was significantly greater than that in the control group ($p < 0.05$). There were no significant differences between the percentages of pulmonary lymphomas in the control group and in the 2 treated groups. However, there was a significant increase in the percentage of nonlymphomatous malignant tumors among rats of Group B ($p < 0.02$). The histological varieties and sites of origin of the nonlymphomatous malignant tumors that were found in this study are listed in the footnotes to Table 1. These tumors were all unequivocally malignant, and distant metastases were present in the majority of the tumor-bearing rats. The histological appearances of selected tumors are illustrated in Figs. 1 to 6.

DISCUSSION

In the present investigation, a significant increase in the incidence of malignant tumors was observed in rats that received 6 i.v. injections of nickel carbonyl. The malignant tumors included a variety of histological types, and developed in diverse organs and tissues. Thus, this study provides additional evidence of the carcinogenicity of nickel carbonyl in the rat. Previous investigations which have demonstrated the carcinogenicity of various nickel compounds in experimental animals have been reviewed by Sunderman (24). Nickel carcinogenesis has been documented in several strains of rats, as well as in mice, guinea pigs, hamsters, rabbits, and cats (24). In rats, the carcinogenic potency of nickel compounds appears to be inversely related to the solubilities of the compounds in aqueous media. Thus, the carcinogenic nickel compounds, which include nickel dust, nickel sulfide, nickel carbonate, nickel oxide, nickel carbonyl, and nickelocene, are all sparingly soluble in water at 37° (8, 14, 24). Soluble nickel salts, such as nickel chloride, nickel sulfate, and nickel ammonium sulfate, have not been found to be carcinogenic (8, 14). The biological characteristics of the malignant tumors that are induced in rats by administration of nickel

Table 2

Resume of acute biochemical effects of i.v. nickel carbonyl in Sprague-Dawley rats

Ni(CO)₄, 2.2 mg/100 g, i.v., 6 to 28 hr before sacrifice.

<table>
<thead>
<tr>
<th>Experimental system</th>
<th>% of control values</th>
<th>p vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic tryptophan pyrrolase after cortisol induction</td>
<td>72 ± 7^b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hepatic benzpyrene hydroxylase after phenothiazine induction</td>
<td>45 ± 8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hepatic cytochrome P-450 after phenobarbital induction</td>
<td>48 ± 5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Leucine-¹⁴C uptake in hepatic microsomal proteins in vivo</td>
<td>82 ± 6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>¹⁴C-Labeled orotic acid uptake in hepatic RNA in vivo</td>
<td>25 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RNA polymerase activity in hepatic nuclei</td>
<td>40 ± 7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RNA synthesis in vitro by hepatic chromatin-RNA polymerase complex</td>
<td>49 ± 6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Template activity of hepatic DNA for M. lysodeikticus RNA polymerase</td>
<td>98 ± 6</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

- References are cited in the text.
- Mean ± S.E.
compounds have been summarized by Sunderland (22) and Maenza et al. (13).

The acute biochemical effects that have been reported to occur in hepatocytes of Sprague-Dawley rats following i.v. injection of nickel carbonyl in LD₅₀ dosage are listed in Table 2. Nickel carbonyl inhibits enzyme induction in several experimental systems, including cortisone induction of tryptophan pyrrolase (20), phenothiazine induction of benzpyrene hydroxylase (19), and phenobarbital induction of cytochrome P-450 (21). There is mild inhibition of protein synthesis, as measured by in vivo incorporation of leucine-14C into hepatic microsomal proteins (23), and there is marked inhibition of RNA synthesis, as measured by in vivo incorporation of 14C-labeled orotic acid into hepatic RNA (2).

The inhibition of RNA synthesis is attributable to nickel carbonyl inhibition of DNA-dependent RNA polymerase activity, as demonstrated in vitro with intact hepatic nuclei (25) and also with chromatin-RNA polymerase complex isolated from hepatic nuclei (3). Nickel carbonyl does not affect the template activity of hepatic DNA or chromatin for transcription in vitro by exogenous RNA polymerase from Micrococcus lysodeikticus (1). The finding that i.v. nickel carbonyl inhibits DNA-dependent RNA synthesis in rat hepatocytes furnishes a metabolic effect whereby nickel carbonyl can cause acute alterations of gene expression. A possible relationship to the mechanism of nickel carbonyl carcinogenesis is suggested by similarities between the acute biochemical alterations produced by nickel carbonyl and those produced by certain nonmetallic carcinogens, such as aflatoxin B₁ (7). Caution should be exercised in interpreting this analogy, however, since aflatoxin B₁ is a very potent liver carcinogen, whereas nickel carbonyl is a relatively weak liver carcinogen.

ACKNOWLEDGMENTS

We are grateful to Dr. Svend W. Nielsen, Dr. Ronald M. Maenza, and Dr. Francis J. C. Roe for consultations regarding the histological classification of certain of the malignant tumors, and to Mrs. Sandra Cullifer for skillful technical assistance.

REFERENCES

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Fig. 1. Malignant lymphoma of lung of a female rat of Group B. A, tumor surrounding a small pulmonary artery. X 100. B, pleomorphic round cells with prominent nuclei. X 250.

Fig. 2. Fibrosarcoma of orbit of a male rat of Group A. A, poorly differentiated primary tumor. X 250. B, pulmonary metastasis of the tumor, with infiltration of alveolar septae by neoplastic cells. X 100.

Fig. 3. Undifferentiated sarcoma of abdominal wall of a female rat of Group B. A, sarcoma cells surrounding degenerating skeletal muscle fibers. X 250. B, pleomorphic cells with hyperchromatic nuclei. X 400.

Fig. 4. Cholangiocarcinoma of liver of a female rat of Group A. A, tumor compressing fatty liver tissue (left corner). X 250. B, neoplastic tubular structures lined by polyhedral cells. X 400.

Fig. 5. Hemangioendothelioma of s.c. tissues of abdomen of a male rat in Group B. A, neoplastic endothelial cells lining vascular channels. X 100. B, prominent vascular spaces. X 100.

Fig. 6. Undifferentiated leukemia in a male rat of Group B. A, focus of small round cells with large hyperchromatic nuclei in the myocardium. X 450. B, focus of undifferentiated round cells in the liver. X 450.
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