Pathological Reactions in Lung, Liver, Thymus, and Spleen of Rats after Subacute Parenteral Administration of Nickel Sulfate*†

JOSEPH A. KNIGHT, M.D.,‡
Marilyn R. PLOWMAN, B.S.§
SIDNEY M. HOPFER, PH.D.,§
and F. WILLIAM SUNDERMAN JR., M.D.§

‡Department of Pathology,
University of Utah School of Medicine,
Salt Lake City, UT 84132
and
§Departments of Laboratory Medicine and Pharmacology,
University of Connecticut School of Medicine,
Farmington, CT 06030

ABSTRACT

Male Fischer-344 rats (10 to 12 rats per group) were given 14 i.m. injection of nickel sulfate (NiSO₄) over a period of 26 days in order to delineate the two-year survival, body weight curves, hematocrit responses, and histopathological reactions, including carcinogenesis. Group A (vehicle controls) received the NaCl vehicle; Groups B, C, and D received NiSO₄ solution at dosages of 63, 83, or 125 μmol per kg, respectively. Rats in Group D all died during the period from 4 to 32 days after the first injection; survival of rats in Groups B and C did not differ significantly from the controls (Group A). No differences were found at any time between the mean body weights or blood hematocrits of the NiSO₄-treated groups vs. the controls. In Group D, histopathological lesions of the lung, liver, thymus, and spleen were consistently observed; these lesions were most severe in rats that died during the period from 22 to 32 days after the first injection. The lungs showed proliferation of alveolar lining cells, thickening of the alveolar wall, and proteinaceous alveolar exudate. The livers showed microvesicular steatosis, and necrotic hepatocytes were scattered throughout the lobules. Degeneration of lymphocytes, with pyknosis and karyorrhexis, was observed in the thymic cortex and the white pulp of the spleen. At necropsy, no significant differences were found between the NiSO₄-treated rats of Groups B and C and the controls (Group A). No
sarcomas or other neoplasms occurred near the injection sites in NiSO₄-treated rats. This study shows that repeated i.m. administration of NiSO₄ to rats cause pulmonary toxicity, hepatic toxicity, and immunotoxicity, but does not induce erythrocytosis or carcinogenesis. If the dose of NiSO₄ is sublethal, the acute tissue damage is evidently healed, so that surviving rats have normal body weight curves and longevity.

Introduction

Several studies in our laboratory have described the acute and subacute toxic reactions that occur in rats following parenteral administration of Ni²⁺. Acute LD₅₀ values for NiCl₂ in rats are 85 μmol per kg (i.p.), 390 μmol per kg (i.m.), and 420 μmol per kg (s.c.). Gitlitz et al⁴ reported that an injection of NiCl₂ (68 μmol per kg, i.p.) induced acute nephropathy, with glomerular damage, focal tubular necrosis, proteinuria, and aminoaciduria. Donskoy et al³ found that an injection of NiCl₂ (500 μmol per kg, s.c.) caused hepatic toxicity, with fatty metamorphosis, hydropic degeneration, focal inflammation, hypertransaminasemia, and elevated hepatic lipoperoxides. Knight et al¹⁶ observed that an injection of NiCl₂ (500 μmol per kg, s.c.) also produced acute thymic involution, with pyknosis and karyorrhexis of cortical lymphocytes, and elevated thymic lipoperoxides. Knight et al¹⁵ showed that daily injections of NiCl₂ (63 or 125 μmol per kg, s.c.) for 3 to 6 weeks caused histopathological reactions in rat lungs, evidenced by proliferation of alveolar lining cells (especially Type II pneumocytes), thickened alveolar walls, proteinaceous alveolar exudate, hydropic degeneration of vascular endothelium, and hyperplastic bronchial epithelium. Oskarsson et al²² noted that continuous infusion of NiCl₂ (80 μmol/kg per day, i.p.) for three weeks did not stimulate erythropoiesis, whereas infusion of CoCl₂ caused marked erythrocytosis at the same dosage.

The objective of the present study was to assess the long-term sequellae (especially regarding longevity and neoplasia) in rats following repeated parenteral injections of Ni²⁺. Pott et al²³ observed abdominal mesotheliomas and fibrosarcomas in rats that were treated twice weekly for six months by i.p. injection of NiCl₂ or NiSO₄ (~80 μmol per kg), whereas Kasprzak et al¹¹ did not observe any local tumors in rats that were treated every second day for one month by i.m. injection of NiSO₄ (~21 μmol per kg). The present study was similar in design to that of Kasprzak et al,¹¹ in order to test whether or not higher i.m. dosages of NiSO₄ might be carcinogenic in rats.

Materials and Methods

The experimental animals were 42 male rats of the Fischer-344 strain* kept in plastic cages with wood-chip bedding within a laminar-flow hood and fed Purina laboratory chow and water ad libitum. Their body weights averaged 200 g (SD ± 13) at the initiation of the study. The rats were divided into four groups (10 to 12 rats/group), treated as follows: Group A (vehicle controls) received 14 i.m. injections (1 mL/kg body wt) of sterile NaCl vehicle solution (0.15 mol per L, pH 6.5) on days 1, 2, 3, 4, 5, 8, 10, 12, 15, 17, 19, 22, 24, and 26. Groups B, C, and D received 14 similar i.m. injections of NiSO₄ (63, 83, or 125 μmol per kg, body

* Harland-Sprague Farms, Madison, WI.
Figure 1. Cumulative mortality curves for rats in the four experimental groups: Group A (n = 10) received 14 i.m. injections of the NaCl vehicle; Group B (n = 10) received 14 i.m. injections of NiSO₄ (63 μmol per kg); Group C (n = 12) received 14 i.m. injections of NiSO₄ (83 μmol per kg); Group D (n = 10) received 4 to 14 i.m. injections of NiSO₄ (125 μmol per kg). The mortality of Group D was significantly greater than Group A (p < 0.01); Groups B and C did not differ significantly from Group A.

The NiSO₄ injection solutions were prepared by dissolving ultrapure nickel sulfate (NiSO₄·6H₂O)† in the NaCl vehicle. The injections were made deep into the thigh muscles, alternating between the hindlegs.

Immediately prior to the initial injection and at 2, 4, 6, 8, 12, and 16 weeks thereafter, blood samples (80 μL) were obtained by incising the tip of each rat's tail with a scalpel. The blood was collected into heparinized microhematocrit capillary tubes and duplicate measurements of hematocrit were performed by high-speed centrifugation. The rats were examined and weighed biweekly. Most rats died spontaneously; some rats were killed when they became too cachetic to obtain food or water. Two untreated control rats, from the same shipment as the test rats, were killed one month after the experiment was begun, in order to prepare reference tissue sections. The experiment was terminated two years after the initial injection. The rats were autopsied and the tissues fixed in buffered formalin; selected tissues were embedded in paraffin, sectioned at four μm, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical tests (standard deviation, Student's t test, Mann-Whitney U test) were performed according to Sachs; experimental results were expressed as means ± SD.

Results

Mortality, Body Weight, and Blood Hematocrit

Cumulative mortality curves for the experimental groups are shown in figure 1. At two years after the initial injection 8 of 10 rats survived in Group A (vehicle controls), 7 of 10 rats survived in Group B (14 injections of NiSO₄, 63 μmol per kg), and 9 of 12 rats survived in Groups C (14 injections of NiSO₄, 83 μmol per kg). In contrast, all 10 rats in Group D died.

† Alfa Inorganics Division, Ventron Corp., Beverly, MA.
within five weeks after the initial injection (p < 0.01 vs. Group A). The rats in Group D died on days 4, 5, 7, 16, 22, 25, 26, 31, and 32 days, respectively; these rats had received 4 to 14 injections of NiSO₄ (125 μmol per kg per injection).

Body weight curves and hematocrit data are shown in figure 2 and figure 3. No significant differences were observed at any time between the mean body weights or hematocrits of the NiSO₄-treated rats in Groups B, C, or D vs. the vehicle controls (Group A).

**Histopathological Reactions in Group D**

Upon gross examination at necropsy, the lungs of rats in Group D were mottled and severely congested; the thymus glands were much smaller than the controls; the other tissues were grossly normal. Sections of lung, liver, kidney, spleen, pancreas, brain, thymus, thyroid, and testis from 7 of 10 rats in Group D were suitable for microscopic study; the tissues of the remaining rats in Group D were unsuitable, owing to post-mortem autolysis. The histopathological changes that are summarized in the following paragraphs were seen in all rats of Group D that were examined. The lesions were relatively mild in rats that died on days 4 to 16 and moderate or severe in rats that died on days 22 to 32 after the first injection of NiSO₄.

**Lungs.** Proliferation of alveolar lining cells and thickening of the alveolar wall were the most prominent findings (figure 4, Panel A). These lesions were present throughout the lungs, but were especially marked at the periphery. Large alveolar macrophages and occasional polymorphonuclear leukocytes were noted in the alveolar spaces, as well as proteinaceous exudate.

**Heart.** The subepicardial venous channels were dilated and filled with blood. Microvacuolar fatty changes were seen in the myocardium, but cellular necrosis was not observed.

![Figure 2. Body weight curves for rats in the four experimental groups. No significant differences were found at any time between the mean body weights of NiSO₄-treated rats in Groups B, C, or D and the corresponding value in the controls (Group A). The error bars show typical standard deviations for body weights in the various groups.](image-url)
Liver. Necrotic hepatocytes were scattered throughout the hepatic lobules (figure 4, Panel B). The livers were generally congested and showed microvesicular steatosis of non-necrotic hepatocytes. There was little or no inflammatory response around necrotic cells. Binucleate hepatocytes were common.

Thymus. Compared to untreated controls, the corticomedullary junction of the thymus was indistinct; there was extensive degeneration and depletion of lymphocytes in the thymic cortex, with nuclear pyknosis and karyorrhexis (figure 4, Panel C). There was also histiocytic phagocytosis.

Spleen and Lymph Nodes. The spleens were moderately congested. Lymphocytes were focally depleted in the white pulp and showed nuclear pyknosis and fragmentation (figure 4, Panel D). Similar degenerative changes were seen in lymphocytes scattered throughout the red pulp, although these changes were less conspicuous. Histiocytic phagocytosis was occasionally noted. A mediastinal lymph node and a retroperitoneal lymph node showed lymphocytic pyknosis and karyorrhexis.

Other tissues. The kidneys generally showed vascular congestion; microvacuolation of proximal convoluted tubules was evident in one kidney. No significant abnormalities were observed in the thyroid gland, pancreas, testes, or brain of rats in Group D.

Carcinogenesis Bioassay

No sarcomas or other neoplasms developed within two years in proximity to the injection sites in vehicle controls (Group A) or NiSO₄-treated rats (Groups B and C). The only tumor was a subcutaneous fibroma in the neck of a rat in Group C. At necropsy, no significant differences were
found between the NiSO₄-treated rats (Groups B and C) and the vehicle controls (Group A).

Discussion

This study shows that repeated i.m. administration of NiSO₄ to rats causes pulmonary toxicity, hepatic toxicity, and immunotoxicity. The rats in Group D died within five weeks after the initial i.m. injection of NiSO₄; their lung lesions closely resembled those previously noted in rats after daily s.c. injections of NiCl₂ for three to six weeks. The signs of immunotoxicity in these rats were similar to those observed in the thymus of rats after a single injection of NiCl₂. The signs of hepatic toxicity mimicked those reported after single or multiple injections of NiCl₂ or NiSO₄. This study also showed that i.m. administration of NiSO₄ did not induce erythrocytosis in rats, although an intrarenal injection of nickel subsulfide (αNi₃S₂) has been shown to stimulate erythropoiesis in rats by enhancing erythropoietin production, and erythrocytosis has been observed in humans who accidentally ingested a solution of NiCl₂ and NiSO₄.

An important outcome of this study was the demonstration that body weight curves and long-term survival of rats in Groups B and C, which received 14 i.m. injections of NiSO₄ at dosages of 63 or 83 μmol per kg, did not differ significantly from the corresponding data for the vehicle controls (Group A). No long-term sequelae of the NiSO₄ exposures were detected. The repeated i.m. administration of NiSO₄ to rats failed to induce any sarcomas at the injection sites or to increase the overall tumor incidence. The negative outcome of the present carcinogenesis bioassay was consistent with previous findings of Kasprzak et al, who tested a lower dosage of NiSO₄ (~21 μmol per kg, i.m., ×15) in a similar regimen. The positive carcinogenesis tests of NiCl₂ and NiSO₄ that were reported by Pott et al involved a different parenteral route (i.p. instead of i.m.), longer treatment period (six months instead of one month), and longer observation period (30 months instead of 24 months).

The time-course, tissue-specificity, and dose-response relationships for the toxic reactions in the NiSO₄-treated rats were consistent with recent evidence that acute Ni²⁺-toxicity is mediated by oxygen free-radical mechanisms, such as lipid and DNA peroxidation. In the presence of histidyl peptides and hydroperoxide, Ni²⁺ is a potent catalyst for hydroxyl radical generation. Tissue targets of Ni²⁺-toxicity (e.g., lung, thymus, liver, kidney) accumulate malondialdehyde and other peroxidation products and display impaired defenses against oxidant injury. Autocatalytic peroxidation induced by Ni²⁺ appears to be a threshold phenomenon that is temporally delimited and occurs only at high dosages or tissue concentrations of Ni²⁺. The present study suggests that, if the dose of Ni⁺ is sublethal, the acute tissue damage can be healed, so that the surviving rats have normal body-weight curves and longevity.

For background information about the toxicity and carcinogenicity of nickel compounds, readers may consult recent reviews and monographs.

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**Figure 4.** Photomicrographs (hematoxylin-eosin stain; magnification ×328) of tissue sections from a rat in Group D that died on day 26, after receiving 14 i.m. injections of NiSO₄ (125 μmol per kg). Panel A: Lung, showing hyperplasia of alveolar lining cells and thickening of the alveolar wall. Panel B: Liver, showing necrosis of scattered hepatocytes and focal microvesicular steatosis. Panel C: Thymus, showing degeneration of cortical lymphocytes with prominent pyknosis and karyorrhexis. Panel D: Spleen, showing typical pyknosis and karyorrhexis of lymphocytes in the white pulp.
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References


