Hepatic Toxicity of Nickel Chloride in Rats*†

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ABSTRACT

Enhanced lipid peroxidation was observed in livers of rats killed 24 hr after sc injection of nickel chloride (NiCl₂) (750 μmol per kg), as evidenced by 13-fold increase of conjugated dienes in microsomal lipids and 4-fold increase of thiobarbituric acid (TBA) chromogens in hepatic cytosol. Histologic examination of livers from rats killed one to three days after NiCl₂ injection (500 μmol per kg) showed microvesicular fatty metamorphosis, mild hydropic degeneration, and foci of inflammation. Microvesicular steatosis of hepatocytes was confirmed by electron microscopy. Dose-related increases of serum aspartate aminotransferase (AST) activity (up to 7-fold vs controls) and alanine aminotransferase (ALT) activity (up to 3-fold vs controls) were observed 24 hr after injection of NiCl₂ (125 to 750 μmol per kg); diminished serum alkaline phosphatase activity (up to 72 percent reduction vs controls) was seen at NiCl₂ dosages from 375 to 750 μmol per kg. Diethyldithiocarbamate did not influence the effects of NiCl₂ on TBA-chromogens in liver homogenates or on serum AST and ALT activities but acted synergistically with NiCl₂ to diminish serum alkaline phosphatase activity and to increase serum bilirubin concentration. This study demonstrates that parenteral administration of NiCl₂ to rats produces acute hepatic toxicity, with enhanced lipid peroxidation, microvesicular steatosis, and increased serum AST and ALT activities.

Introduction

The toxicity and carcinogenicity of nickel compounds in experimental animals and man have been reviewed in recent monographs.5,17 Numerous investigators have studied the toxic effects of nickel compounds on the kidney, lung, heart, and endocrine glands, as well as the fetus, but the hepatic toxicity of nickel compounds has been relatively neglected. Sunderman et al21 showed that parenteral administration of NiCl₂ to rats enhances hepatic lipid peroxidation, based upon measurements of thiobarbituric acid (TBA) chromogens in liver homogenates. This study corroborated...
the observation of Fawade and Pawar\(^7\) that hepatic microsomes from NiCl\(_2\)-treated rats produce increased amounts of TBA-chromogens, when incubated in vitro in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or ascorbate. The present paper describes further studies of NiCl\(_2\) hepatotoxicity in rats, including (a) centrifugal fractionations of TBA-chromogens in liver homogenates; (b) measurements of conjugated dienes in lipids extracted from hepatic microsomes; (c) histologic examinations of liver by light and electron microscopy; (d) assays of serum aminotransferase and alkaline phosphatase activities; (e) determinations of serum bilirubin and nickel concentrations; and (f) tests of the effects of diethylldithiocarbamate, a Ni\([\text{II}\]]\)-chelator. These biochemical and morphological studies demonstrate that a single parenteral injection of NiCl\(_2\) can produce acute, dose-related hepatocellular damage in rats.

**Materials and Methods**

The test substances were ultrapure nickel chloride (NiCl\(_2\)·3H\(_2\)O) and sodium diethylldithiocarbamate (DDC), recrystallized according to Baselt et al.\(^1\) The experimental animals were 302 male rats of the Fischer-344 strain (body wt. = 190 to 260 g), fed Purina laboratory chow and water ad libitum. Control rats were treated with NaCl vehicle solution (140 mmol per L, 0.2 to 0.3 ml per rat), by sc and/or im injections at the same time that test rats received injections of NiCl\(_2\) (125 to 750 \(\mu\)mol per kg, body wt., sc) and/or diethylldithiocarbamate (1.0 or 1.3 mmol per kg, body wt., im).* Food was removed from the cages 17 hr prior to sacrifice. Blood samples were obtained by cardiac puncture or aspiration from the retro-orbital venous plexus under ether anesthesia. Liver homogenates (one g of liver to four ml of medium) were prepared with a motor-driven Teflon-glass tissue homogenizer.

For measurements of TBA-chromogens, the homogenization medium was KCl solution (154 mmol per L, 4\(^\circ\)C); for measurements of conjugated dienes in microsomal lipids, the homogenization medium was phosphate-KCl-EDTA solution (KH\(_2\)PO\(_4\), 20 mmol/L; NaOH, 15.7 mmol/L; KCl, 134 mmol/L; ethylenediamine tetraacetic acid (EDTA), one mmol per L; pH 7.4; 4\(^\circ\)C). The homogenates were filtered through fine-mesh plastic gauze and centrifuged at 18,000 \(g\) for 15 min at 4\(^\circ\)C to sediment nuclei, mitochondria, and cell debris; the supernates were centrifuged at 105,000 \(g\) for 60 min at 4\(^\circ\)C to sediment microsomes.

The following analytical methods were used: (a) measurement of hepatic dry weight;\(^21\) (b) assay of TBA-chromogens;\(^21\) with results expressed as nmol malondialdehyde (MDA) per g (dry wt.) of liver; (c) extraction of microsomal lipids and spectrophotometric assay of conjugated dienes;\(^15\) (d) measurement of microsomal lipid concentrations by dichromate oxidation;\(^4\) (e) kinetic assays of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities\(^3\) and serum alkaline phosphatase activity;\(^25\) (f) determination of serum bilirubin by the diazo reaction;\(^14\) and (g) analysis of serum nickel by electrothermal atomic absorption spectrophotometry with Zeeman background correction.\(^19\)

For histologic examination by light microscopy, liver was fixed in 10 percent neutral buffered formalin; tissue sections were stained with hematoxylin and eosin and by the periodic-acid-Schiff (PAS) technique (with and without diastase...
treatment). For electron microscopy, liver was diced in buffered glutaraldehyde at 0°C, post-fixed in buffered osmium tetroxide, embedded in plastic, stained with uranyl acetate-lead citrate, and examined by use of a Philips-300 electron microscope at 60 kV.

Statistical tests (standard deviation, paired and non-paired t tests) were performed according to Rosner; experimental results are given as means ± SD.

Results

Lipid Peroxidation

Six pairs of rats were killed 24 hr after sc injection of NiCl₂ (750 μmol per kg) or NaCl vehicle solution (controls). Livers of NiCl₂-treated rats were enlarged and congested, with increased wet weights (liver weight = 7.1 ± 1.0 g in NiCl₂-treated rats, P < 0.01 versus 5.5 ± 0.8 g in paired controls) and increased liver:body weight ratios (liver weight = 3.2 ± 0.3 percent of body weight in NiCl₂-treated rats, P < 0.01 versus 2.5 ± 0.2 percent in paired controls). Hepatic dry weights averaged 34.0 ± 0.5 percent of wet weight in NiCl₂-treated rats, which did not differ significantly from the corresponding values (33.9 ± 1.0 percent) in paired controls. Consistent with previous findings, the mean concentration of TBA-chromogens was increased 2.3-fold in liver homogenates from NiCl₂-treated rats (figure 1, left panel). As shown in the center panel (figure 1), concentrations of TBA-chromogens were (a) increased 2-fold in the 18,000 g sediment, which contained nuclei, mitochondria, and cell debris; (b) not significantly increased in the microsomal pellet; and (c) increased 4-fold in the cytosol (105,000 g supernate). Conjugated dienes in microsomal lipids were increased 13-fold, compared to paired controls (figure 1, right panel). Spectrophotometric scans of microsomal lipids from paired NiCl₂-treated and control rats are illustrated in figure 2, together with the characteristic difference spectrum of conjugated dienes (absorption maximum = 233 nm).

![Figure 1](image-url)

NiCl₂-treated rats are expressed as multiples of paired values obtained by simultaneous analyses of 18,000 g sediment, 105,000 g sediment, and 105,000 g supernate from liver homogenates of the six NaCl-treated control rats. Right Panel: Concentrations of conjugated dienes in lipid extracts of hepatic microsomes from six NiCl₂-treated and six control rats.
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**Figure 2.** Diene conjugation absorption spectra of lipid extracts of hepatic microsomes from a NiCl₂-treated rat (750 μmol per kg, sc, 24 hr before sacrifice) and a paired control rat. The lipid concentrations were 0.5 mg per ml of cyclohexane. **Left Panel:** Spectrophotometric curves obtained with lipid extracts of hepatic microsomes in the sample cuvette and cyclohexane in the reference cuvette. **Right Panel:** Difference spectrum obtained by subtracting the absorption curve of hepatic microsomal lipids from a control rat from the corresponding curve of lipids from a paired NiCl₂-treated rat.

**Histopathological Findings**

In a serial-sacrifice study, 12 rats received sc injection of NiCl₂ (500 μmol per kg) and four rats received sc injection of NaCl vehicle solution (controls). Three NiCl₂-treated rats and one control rat were killed at each of the following intervals post-injection; 6, 24, 48, and 72 hr. On light microscopic examination, livers from control rats appeared normal. The most notable finding in livers from NiCl₂-treated rats was microvesicular fatty metamorphosis, which was focal at six hr post-injection and became progressively more prominent at 24, 48, and 72 hr (figure 3). Other morphologic findings in livers from NiCl₂-treated rats included (a) mild hydropic degeneration, (b) variable congestion, and (c) scattered foci of inflammation. There was no evidence of hepatic necrosis or cholestasis. Small deposits of PAS-positive, diastase-resistant material were noted in Kupffer cells. This PAS-positive material, which may represent cytoplasmic proteins released from damaged hepatocytes, was evident at 24 to 72 hr post-injection in NiCl₂-treated rats but was also seen, to a lesser degree, in control rats. Electron microscopy confirmed the presence of microvesicular steatosis in hepatocytes of NiCl₂-treated rats (figure 4).

**Serum Enzyme and Bilirubin Assays**

A dose-effect study of serum enzyme activities was performed in six test groups (5 to 18 rats per group) that received sc injections of NiCl₂ at dosages of 125, 250, 375, 500, 625, or 750 μmol per kg and in a control group (14 rats) that received sc injection of the NaCl vehicle solution; the rats were all killed 24 hr post-injection. As shown in figure 5, significant increases of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were observed in all NiCl₂-treated rats. The increases were dose-related; the maximal (7-fold) increase of serum AST activity and the maximal (3-fold) increase of serum ALT activity occurred at the 625 μmol per kg dosage of NiCl₂.

In a time-course study, six test groups (6 to 15 rats per group) were killed 3, 6, 18, 24, 48, or 72 hr after sc injection of NiCl₂ (500 μmol per kg); six control groups (6 to 7 rats per group) were killed at corresponding intervals after sc injection of NaCl vehicle solution. Since analytical results in the control groups did not differ significantly, the results in the 41 control rats were combined. Significant increases of serum AST and ALT activities were observed in NiCl₂-treated rats at all of the intervals post-injection (figure 6). The time-courses of increased serum AST and ALT activities were parallel, with maximal (7-fold) increase of serum AST activity and maximal (3-fold) increase of serum ALT activity at 24 hr after injection of NiCl₂.

Dose-effect and time-course relationships for serum alkaline phosphatase activity in the same groups of NiCl₂-
treated rats are shown in figure 7. Significant diminution of serum alkaline phosphatase activity was noted at 18 to 72 hr after injection of NiCl₂ (500 μmol per kg) (figure 7, upper panel). Dose-related diminution of serum alkaline phosphatase activity was observed in rats killed 24 hr after injection of NiCl₂ at dosages from 375 to 750 μmol per kg (figure 7, lower panel). At the highest dosage of NiCl₂ (750 μmol per kg), there was 72 percent reduction of serum alkaline phosphatase activity.

Nickel chloride was added in concentrations of 10, 100, 250, and 500 μmol per L to serum specimens from 6 control rats. These in vitro additions of NiCl₂ did not cause significant alterations of serum alkaline phosphatase, AST, or ALT activities, indicating that abnormalities of serum enzyme activities in NiCl₂-treated rats are not mediated by direct inhibition or activation of the enzymes by serum Ni[II].

Bilirubin concentrations in serum specimens from control rats in the dose-effect and time-course studies averaged 0.5 ± 0.4 mg per L. No significant increases of serum bilirubin concentrations were noted in rats killed after injections of NiCl₂ at dosages from 125 to 750 μmol per kg; the mean values for
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Figure 5. Dose-effect study of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in sera from rats at 24 hr after sc injection of NaCl vehicle (controls, N = 14) or NiCl$_2$ (125 to 750 µmol/kg, 5 to 18 rats per group).

Figure 6. Time-course study of AST and ALT activities in sera from control rats (N = 41) and NiCl$_2$-treated rats (500 µmol/kg, sc, 6 to 15 rats/group).

Figure 7. Alkaline phosphatase activity in sera from the same groups of control and NiCl$_2$-treated rats that are described in the captions for figures 5 and 6.

Serum bilirubin concentrations ranged from 0.6 ± 0.8 to 1.1 ± 0.9 mg per L.

**Effects of Diethyldithiocarbamate**

Sodium diethyldithiocarbamate (DDC), was administered to rats by im injection, alone, or with simultaneous sc injection of NiCl$_2$, at dosage combinations that have been shown to cause 6-fold increase of hepatic nickel concentration and 6-fold increase of hepatic heme oxygenase activity, compared to rats that received NiCl$_2$, alone. As shown in table I, serum nickel concentrations were increased 3-fold in rats killed 24 hr after injection of DDC, alone, compared to vehicle controls; serum nickel concentrations were increased 1.4-fold in rats killed 24 hr after simultaneous injections of DDC and NiCl$_2$, compared to rats that received only NiCl$_2$.

At 24 hr after administration of DDC, alone, serum AST activity was increased 2.5-fold and serum ALT activity was increased 1.3 fold, compared to vehicle-treated controls. Administration of DDC plus NiCl$_2$ caused statistically insignificant increases of serum AST and ALT activities, compared to rats that received only NiCl$_2$ (figure 8, upper panels). Administration of DDC and NiCl$_2$, singly, at the specified dosages did not affect serum alkaline phosphatase activ-
TABLE I

<table>
<thead>
<tr>
<th>NiCl₂ (μmol/kg)</th>
<th>DDC (mmol/kg)</th>
<th>No. of Rats</th>
<th>Serum Ni, mean ± SD (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>250</td>
<td>0</td>
<td>9</td>
<td>3,900 ± 1,900</td>
</tr>
<tr>
<td>0</td>
<td>1.3</td>
<td>8</td>
<td>7.0 ± 2.9†</td>
</tr>
<tr>
<td>250</td>
<td>1.3</td>
<td>10</td>
<td>5,400 ± 1,500$</td>
</tr>
</tbody>
</table>

*Nickel chloride (NiCl₂) injected sc; Diethyldithiocarbamate (DDC) injected im; 24 hours before death.
†p < 0.05 vs vehicle-treated controls.
$ p < 0.05 vs NiCl₂ alone.

Administration of DDC, alone, caused 50 percent increase of hepatic concentration of TBA-chromogens, compared to vehicle-treated controls; administration of DDC plus NiCl₂ did not significantly affect hepatic concentration of TBA-chromogens, compared to rats that received only NiCl₂ (table II).

Discussion

The present findings that conjugated dienes are increased 13-fold in hepatic microsomal lipids and that TBA-chromogens are increased 4-fold in hepatic cytosol of NiCl₂-treated rats implicate lipid peroxidation as a possible molecular mechanism for hepatocellular injury in acute Ni[II]-poisoning. The time-course and dose-effect relationships for NiCl₂-induced increases of serum AST and ALT activities, as observed in the present study, correspond closely to the previously reported time-course and
TABLE II
Effect of Diethyldithiocarbamate on Hepatic TBA-Chromogen Concentration in NiCl₂-Treated Rats*

<table>
<thead>
<tr>
<th>NiCl₂ (μmol/kg)</th>
<th>DDC (μmol/kg)</th>
<th>No. of Rats</th>
<th>TBA-Chromogens mean ± SD (n mole MDA/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.86 ± 0.30</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>7</td>
<td>2.14 ± 0.82f</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>6</td>
<td>1.32 ± 0.75f</td>
</tr>
<tr>
<td>500</td>
<td>1.0</td>
<td>6</td>
<td>2.05 ± 0.74f</td>
</tr>
</tbody>
</table>

*Nickel chloride (NiCl₂) injected sc; Diethyldithiocarbamate (DDC) injected im; 18 hours before death.
†p < 0.05 vs vehicle-treated controls.
§Not significantly different from NiCl₂, alone.

dose-effect relationships for NiCl₂-induced increases of TBA-chromogens in liver homogenates. Hepatic lipid peroxidation in NiCl₂-treated rats may reflect the transient depletion of hepatic glutathione that occurs four to six hr after injection of NiCl₂. In considering the pathophysiological significance of lipid peroxidation, Younes and Siegers, Halliwell and Gutteridge, and Clavel et al delineated three general cases: (1) lipid peroxidation that represents an end-result of cell necrosis and autolysis, (2) lipid peroxidation that induces cell injury by a cascade of subsequent events, including lysosomal enzyme release and intracellular Ca²⁺ uptake, and (3) lipid peroxidation that is inhibited or reversed by protective cellular systems, such as glutathione peroxidase, and by endogenous and exogenous antioxidants. The absence of histologic evidence of hepatic necrosis in NiCl₂-treated rats militates against accumulation of TBA-chromogens and lipid conjugated dienes as end-results of hepatocellular demise. To elucidate the role of lipid peroxidation in the hepatic toxicity of NiCl₂, studies are underway to determine whether or not Ni(II)-induced lipid peroxidation and hepatocellular damage are suppressed by administration of antioxidants.

In the present study, the morphological changes in livers of NiCl₂-treated rats (500 μmol per kg, sc) were less pronounced than those observed by Mathur et al following daily administration of NiSO₄ to rats for one to three months. Mathur et al noted foamy cytoplasm of hepatocytes and focal hepatic necrosis in rats that received 60 or 90 daily injections of NiSO₄ (50 μmol per kg, ip); periportal hypercellularity, bile duct proliferation, and Kupffer cell hyperplasia were evident at 90 days. Mathur et al also detected foamy cytoplasm and vacuolization of hepatocytes, congestion and dilatation of hepatic sinusoids, and focal necrosis of hepatic parenchyma in rats that received dermal applications of NiSO₄ (1.0 and 1.7 mmol per kg, daily, for one month). On the other hand, Yawets et al did not report morphological changes in livers of rats that received six injections of NiCl₂ (170 μmol per kg, ip, thrice weekly for two weeks), although the NiCl₂ treatments suppressed phenobarbital induction of hepatic cytochrome P-450.

The dose-related increases of serum AST and ALT activities that occurred in NiCl₂-treated rats have not previously been reported. Hepatocellular release is probably responsible for increased serum activities of these enzymes, but toxic effects of NiCl₂ in extrahepatic tissues may contribute to the observed abnormalities. An unexpected outcome of the present study was the finding that serum alkaline phosphatase activity was diminished in rats that received large doses of NiCl₂. Review of the literature disclosed that such hypophosphatasemia was previously noted by Whanger who found 45 percent reduction of plasma alkaline phosphatase activity in rats fed a diet containing nickel acetate (1000 ppm) for six weeks, and by Weischer et al who observed 21 percent reduction of serum alkaline phosphatase activity in rats exposed continuously to inhalation...
of nickel oxide aerosol (0.8 mg per m$^3$) for 28 days. The mechanism whereby Ni[II] induces hypophosphatasia is unknown.

The influence of diethyldithiocarbamate (DDC) on hepatic lipid peroxidation, serum nickel and bilirubin concentrations, and serum enzyme activities was investigated in view of the synergistic effects of DDC and NiCl$_2$ on heme oxygenase activity and metallothionein concentration in rat liver and other tissues of rats treated with DDC plus NiCl$_2$. In the present study, administration of DDC increased serum nickel concentrations in control and NiCl$_2$-treated rats, but did not influence the effects of NiCl$_2$ on serum AST and ALT activities or hepatic concentrations of TBA-chromogens. Combined injections of DDC and NiCl$_2$ acted synergistically to diminish serum alkaline phosphatase activity and to increase serum bilirubin concentration. The latter finding probably reflects synergistic induction of heme oxygenase activity, since heme oxygenase is the rate-limiting enzyme in the pathway of heme degradation to bilirubin.

Acknowledgments

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References


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