Acute Lethal Toxicity, Hyperkalemia Associated with Renal Injury and Hepatic Damage after Intravenous Administration of Cadmium Nitrate in Rats

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Abstract: Acute Lethal Toxicity, Hyperkalemia Associated with Renal Injury and Hepatic Damage after Intravenous Administration of Cadmium Nitrate in Rats: Emi Dote, et al. Department of Hygiene and Public Health, Osaka Medical College—Cadmium nitrate Cd(NO₃)₂ (CdN) is commonly used in Ni-Cd battery factories. The possibility of accidental exposure to CdN is great. CdN is very soluble in water compared to other Cd compounds. Therefore, acute toxicity would be expected to be quick due to rapid absorption after exposure. However, the mechanisms of CdN toxicity have not been fully elucidated. We investigated the acute lethal toxicity and harmful systemic effects of acute exposure to large doses of CdN. The lethal dose and dose-response study of the liver and kidney were determined after intravenous administration of CdN in rats. The LD₅₀ of CdN was determined to be 5.5 mg/kg. Doses of 2.1, 4.2, 6.3 mg/kg were selected for the dose-response study. Liver injury was induced at doses greater than 4.2 mg/kg. Severe hepatic injury occurred in the 6.3 mg/kg group, which would have been caused by acute exposure to the high concentration of Cd that exceeded the critical concentration in hepatic tissue. A remarkable decrease in urine volume in the 6.3 mg/kg group indicated acute renal failure. A decrease in creatinine clearance suggested acute glomerular dysfunction at doses greater than 4.2 mg/kg. Increases in urinary N-acetyl-β-D-glucosaminidase/creatinine, β₂-microglobulin and glucose in the 6.3 mg/kg group indicated proximal tubular injury. Secretion of K ion was also severely affected by proximal tubular injury and severe decreases in urine volume, and an increase in serum K ion was identified at doses greater than 4.2 mg/kg. Thus severe hyperkalemia might be associated with the cardiac-derived lethal toxicity of CdN.

Key words: Cadmium nitrate, Glomerular dysfunction, Proximal tubular injury, Hyperkalemia

Cadmium (Cd) is a vital component of modern technology, with countless applications in the electronics, communications, power-generation and aerospace industries. Cd compounds are produced commercially, and the production and consumption of these compounds are increasing, in particular in China, Korea and Japan. The worldwide production of Cd was approximately 17,200 tons in 2004. In recent years, the uses of Cd have changed. For example, the high demand for Cd in Ni-Cd batteries has more than offset declining uses in pigments, plating and stabilizers which use cadmium chloride (CdL). Ni-Cd battery manufacture currently consumes more than 70% of the global Cd output, and it is expected that this application will increase with the increasing use of rechargeable batteries and potential Ni-Cd battery use in electric vehicles. In Ni-Cd factories, cadmium nitrate (CdN) is commonly used. Solid CdN exists as Cd(NO₃)₂ · 4H₂O and is a white, odorless crystal of molecular weight 236.43 daltons. Its solubility is 215 g/100 ml in water. Its deliquescence is large, and it has a specific gravity of 2.46 g/cc, boiling point of 132°C (270°F), and melting point of 59.5°C (140°F). The aqueous solubility of CdN is more than that of other Cd compounds such as CdL (0°C, 90 g/100 ml), cadmium oxide (CdO) (insoluble in water) and Cd sulfate (CdS) (76 g/100 ml). There is a great possibility of accidental occupational exposure to CdN. Because CdN dissolves easily in water, it is quickly absorbed upon inhalation or ingestion. There have been many animal studies on acute hepato- and nephro-toxicity caused by CdL and on acute lung disorders caused by CdO. It is believed that each Cd compound, such as CdL, CdO and CdN, has specific...
harmful effects because they have different chemical forms and characteristics. However, the mechanisms of mortality and acute specific effects of CdN on target organs such as the liver and kidney have not been well characterized. In this study, we investigated the acute lethal toxicity and harmful systemic effects of exposure to large doses of CdN.

Materials and Methods

Animals and diets

Ten-week-old SPF male Sprague-Dawley rats weighing 300–350 g were obtained from Japan SLC (Shizuoka, Japan). Animals had free access to rat chow (Funabashi Farm MM-3; Chiba, Japan) and tap water, and they were housed in a separate room at a constant temperature (22.0 ± 1.0°C) under a 12-h light/dark cycle. All aspects of these studies were conducted under the guidelines of Osaka Medical Ethical Association for Accreditation of Laboratory Animal Care.

Chemicals

CdN solutions (2 mg/ml) were prepared by dissolving Cd(NO₃)₂·4H₂O (99% pure; Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 0.9% saline. The pH of the solution was 6.3.

Lethal dose study

Thirty rats were assigned to one of six exposure groups (n=5). All rats were anesthetized with pentobarbital. Six doses of CdN (2.8, 4.2, 4.3, 4.4, 5.8 or 6.3 mg/kg) were injected through the tail vein by syringe pump (model PHD 200P; Harvard Apparatus, Inc., MA, USA) for 5 min. A probit dose-mortality curve was created based on mortality at 24 h⁰, and the lethal dose of CdN was determined with the use of SPSS software (Chicago, IL).

Dose-response study with blood samples

The maximum dose was set at 6.3 mg/kg (LD₉₀). Doses of 2.1, 4.2 and 6.3 mg/kg were selected for the dose-response study; the ratios of the doses were 1:2:3. The doses of Cd were 1.0, 2.0 and 3.0 mg/kg, respectively. Saline was used as a control. Nitric acid was used to investigate the effect of nitric acid ion. The dose of nitric acid was equal to that of nitric acid ion in 6.3 mg/kg CdN. Twenty-five 10-wk-old SPF male Sprague-Dawley rats were assigned to one of five exposure groups (n=5). All rats were anesthetized with pentobarbital. Rats were injected with CdN (2.1, 4.2 or 6.3 mg/kg), saline or nitric acid. Blood samples were obtained from the right carotid artery 5 hours after infusion. Hepatic injury and renal dysfunction were assumed to have occurred by this time. The following serum substances were measured according to standard protocols recommended by assay suppliers: aspartate aminotransferase (AST, ultraviolet method), alanine aminotransferase (ALT, ultraviolet method), mitochondrial aspartate aminotransferase (m-AST, MDH-ultraviolet method), lactate dehydrogenase (LDH, ultraviolet method), LDH isozyme (agarose gel electrophoresis cataphoresis method), glucose (glucose oxidase method), lactate (enzymatic method, CNP-G7) and pyruvate (enzymatic method, CNP-G7). Liver function was determined according to these plasma enzyme activities. Renal function was determined according to blood urea nitrogen (BUN, urease-glutamic dehydrogenase method) and creatinine (Cr, Jaffe method).

Other systemic harmful effects were determined according to the following: sodium (Na-ion selective electrode method), potassium (K-ion selective electrode method), chloride (Cl-ion selective electrode method), pH, PCO₂, PO₂, HCO₃⁻, O₂ saturation (O₂ SAT) and base excess (BE) (288 Blood Gas System; Bayer, Osaka, Japan). Alveoloarterial oxygen difference (A-aDO₂) values were also calculated.

Dose-response study with urine samples

Twenty 10-wk-old SPF male Sprague-Dawley rats were assigned to one of four groups (n=5). All rats were anesthetized with pentobarbital. CdN (2.1, 4.2 or 6.3 mg/kg) or saline was injected as described above. Nitric acid was not used in this study because there were no significant differences between the saline and nitric acid groups in the blood sample study. After injection, saline was administered for 2 h (3 ml/h) to ensure adequate urine volume. Urine accumulated for 5 h after injection and was obtained from an indwelling catheter (18-G indwelling needle; Terumo Corp., Tokyo, Japan). Catheters were inserted at an angle of 20°. The following urinary variables were measured: volume, relative density, Cd, K, Na, glucose, albumin, pH, creatinine, creatinine clearance (Ccr), β₂-microglobulin (LX test; Eiken Chemical Co. Ltd., Tokyo, Japan), N-acetyl-β-D-glucosaminidase (NAG; Shionogi & Co. Ltd., Osaka, Japan), excreted fraction of filtered sodium (FE Na: (urine Na/plasma Na)/(urine Cr/plasma Cr) × 100), excreted fraction of filtered potassium (FE K: (urine K/plasma K)/(urine Cr/plasma Cr) and NAG/Cr (the value of NAG was corrected for Cr because it was apparently affected by urine volume). Urinary Cd concentration was analyzed by atomic absorption spectrometry (AAS 180–80; Hitachi, Ltd., Tokyo, Japan). Total activities and weights were calculated, taking into consideration the variations in urine volume.

Statistical analysis

Data are expressed as mean ± SD. Statistical analyses were performed with SPSS software. p<0.05 was considered statistically significant. Overall differences between groups were determined by one-way ANOVA. If the one-way ANOVA was significant, differences between individual groups were estimated by Fisher’s
Results

All rats in the 4.2 mg/kg group survived longer than 24 h after injection, and all rats in the 6.3 mg/kg group died. According to the dose-mortality curve, the LD<sub>10</sub> and LD<sub>90</sub> for CdN were determined to be 5.5 mg/kg and 6.3 mg/kg, respectively (Fig. 1). Compared to the saline group, serum AST and ALT were not significantly increased in the 2.1 mg/kg group, but were dose-dependently increased in the 4.2 and 6.3 mg/kg groups. Their mean values were more than 300 IU/L in the 4.2 mg/kg group, respectively, and over 600 IU/L in the 6.3 mg/kg group. m-AST and LDH were increased slightly in the 2.1 and 4.2 mg/kg groups but were increased markedly in the 6.3 mg/kg group (Fig. 2). The LDH<sub>5</sub> isozyme was increased significantly in the 6.3 mg/kg group (data not shown). Serum BUN and Cr were dose-dependently increased (Fig. 3). Serum K ion was dose-dependently increased with a significant increase in the 6.3 mg/kg group compared to the other dosage groups. Serum Na ion and Cl ion were in the reference range in all groups. There were no apparent differences in anion gap between groups. Serum lactate, pyruvate or glucose did not differ significantly between groups. PCO<sub>2</sub>, HCO<sub>3</sub>− and BE were dose-dependently decreased. pH, PO<sub>2</sub>,

protected least-significant difference (LSD) test.

Fig. 1. Dose-mortality curve 24 h after single intravenous injection of Cd(NO<sub>3</sub>)<sub>2</sub>. The curve was based on probit regression analysis.

Fig. 2. Dose-response relations of serum hepatic enzyme activities 5 h after single intravenous injections of saline, nitric acid or Cd(NO<sub>3</sub>)<sub>2</sub> (2.1, 4.2 or 6.3 mg/kg). Mean ± SD; n=5; One-way ANOVA; Fisher’s protected LSD; *p< 0.05 vs. saline.
O₂ SAT and A-aDO₂ showed no apparent differences among groups. There were no significant differences in blood biochemical data and blood gas data between the nitric acid group and the saline group (Tables 1 and 2, Fig. 2).

The excretion of K was dose-dependently decreased. In the 6.3 mg/kg group, the excretion of K was approximately four times less than that in the saline group. The excretion of Na was decreased slightly in the 2.1 and 4.2 mg/kg groups, and it was decreased significantly in the 6.3 mg/kg group. Urine glucose was detected markedly in the 6.3 mg/kg group. There was no

Table 1. Serum electrolytes 5 h after single intravenous injection of saline, nitric acid or Cd(NO₃)₂ (2.1, 4.2 or 6.3 mg/kg)

<table>
<thead>
<tr>
<th>Electrolytes (mEq/l)</th>
<th>Saline</th>
<th>Nitric acid</th>
<th>Cd(NO₃)₂ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>144.8 ± 1.8</td>
<td>143.8 ± 1.3</td>
<td>145.2 ± 0.2</td>
</tr>
<tr>
<td>K</td>
<td>4.68 ± 0.05</td>
<td>4.60 ± 0.68</td>
<td>4.86 ± 0.08</td>
</tr>
<tr>
<td>Cl</td>
<td>103.6 ± 0.5</td>
<td>105.6 ± 1.1</td>
<td>104.8 ± 0.4*</td>
</tr>
<tr>
<td>Anion gap</td>
<td>10.8 ± 1.3</td>
<td>12.1 ± 2.3</td>
<td>13.5 ± 1.2</td>
</tr>
</tbody>
</table>

Mean ± SD; n=5; ANOVA; Fisher’s protected LSD; *p<0.05 vs. saline.

Table 2. Blood gas values 5 h after single intravenous injection of saline, nitric acid or Cd(NO₃)₂ (2.1, 4.2 or 6.3 mg/kg)

<table>
<thead>
<tr>
<th>Electrolytes (mmol/l)</th>
<th>Saline</th>
<th>Nitric acid</th>
<th>Cd(NO₃)₂ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42 ± 0.02</td>
<td>7.41 ± 0.05</td>
<td>7.42 ± 0.009</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>43.6 ± 3.9</td>
<td>42.4 ± 5.2</td>
<td>41.7 ± 1.0</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>105.3 ± 8.6</td>
<td>102.2 ± 27.2</td>
<td>124.7 ± 16.6</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/l)</td>
<td>27.6 ± 1.6</td>
<td>26.1 ± 0.76</td>
<td>27.3 ± 0.26</td>
</tr>
<tr>
<td>SAT (%)</td>
<td>92.2 ± 6.5</td>
<td>97.1 ± 1.5</td>
<td>98.2 ± 0.5</td>
</tr>
<tr>
<td>Base Excess (mmol/l)</td>
<td>3.2 ± 1.8</td>
<td>2.4 ± 0.7</td>
<td>3.3 ± 0.3</td>
</tr>
</tbody>
</table>

Mean ± SD; n=5; ANOVA; Fisher’s protected LSD; *p<0.05 vs. saline.

![Fig. 3. Dose-response relations of BUN and creatinine 5 h after single intravenous injection of saline, nitric acid or Cd(NO₃)₂ (2.1, 4.2 or 6.3 mg/kg). Mean ± SD; n=5; One-way ANOVA; Fisher’s protected LSD; *p<0.05 vs. saline.](image-url)
significant difference in the excretion of albumin among groups (Fig. 4). Urine volume was decreased slightly in the 2.1 and 4.2 mg/kg groups, but it was four times less than that of the saline group in the 6.3 mg/kg group. Although the dosage ratio for CdN was 1:2:3, the ratio of excretion of Cd was 1:5:8. There was no significant difference between the saline group and the 2.1 mg/kg group. There were no significant differences in relative densities of urine among groups (data not shown). Urine pH was increased in the 4.2 and 6.3 mg/kg groups. Ccr
Table 3. Urinary variables for 5 h after single intravenous injection of saline or Cd(NO$_3$)$_2$ (2.1, 4.2 or 6.3 mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Cd(NO$_3$)$_2$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>4.0 ± 0.05</td>
<td>3.2 ± 0.03</td>
</tr>
<tr>
<td>Cadmium (ng)</td>
<td>2.7 ± 0.4</td>
<td>4.8 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.6 ± 0.2</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>1.3 ± 0.08</td>
<td>1.3 ± 0.03</td>
</tr>
<tr>
<td>β$_2$-microglobulin (pg)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FE$_{Na}$ (%)</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>FE$_{K}$ (%)</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.8</td>
</tr>
<tr>
<td>NAG/Cr (10$^2$ U/g)</td>
<td>0.33 ± 0.08</td>
<td>0.2 ± 0.05</td>
</tr>
</tbody>
</table>

Mean ± SD n=5; ANOVA; Fisher’s protected LSD; *p<0.05 vs. saline. †p<0.05 vs. 2.1 mg/kg group. Ccr: creatinine clearance, FE$_{Na}$: excreted fraction of filtered sodium. FE$_{K}$: excreted fraction of filtered potassium.

Discussion

The intravenous LD$_{50}$ values of CdO and CdL have been reported to be 25 mg/kg$^{9}$ and 5.5 mg/kg$^{10}$, respectively. The LD$_{50}$ Cd concentrations were 3.4 mg/kg for CdL and 2.6 mg/kg for CdN. Toxicity is affected not only by Cd concentration but also by the form, this is probably related to solubility. The bioavailability of Cd is dependent on the solubility$^{11}$. On acute exposure, the bioavailabilities and the toxicity of more soluble Cd compounds are higher than those of less soluble Cd compounds after rapid absorption in blood by inhalation exposure$^{12}$. CdN is very soluble in water compared to other Cd compounds. Therefore, acute toxicity would be expected to be relatively rapid after exposure.

CdL-induced hepatotoxicity occurs rapidly as evidenced by increased serum aminotransferase activities as early as 1 h after exposure which increased remarkably until 6 h$^{13}$. The biochemical disorders were thought to have occurred by 5 h after injection of CdL. Given the increases in AST, ALT and LDH, liver injury was induced at doses greater than 4.2 mg/kg (2.0 mg/kg Cd). In the present study, mean values of AST and ALT were more than 600 IU/L in the 3.0 mg/kg Cd (LD$_{50}$ CdN) group 5 h after injection. Marked increases in AST and ALT activities in rats at CdL, doses greater than 1.9 mg/kg Cd were reported, and mean values of AST and ALT 5 hours after intravenous injection of 3.9 mg/kg Cd (LD$_{50}$ CdL) were 700 IU/L$^{13}$. The grade of liver injury likely increases with Cd dose. The nitric acid ion in CdN appears to have little toxic effects. Tzirogiannis et al.$^{14}$ reported that serum AST and ALT levels increase for 10 h after injection of CdL. It was supported that AST and ALT was increased five hours after injection. m-AST is an indicator of mitochondrial injury and is important in the clinical assessment of severe liver injury$^{15}$. The remarkable increase of m-AST indicated that severe liver injury occurred in the 6.3 mg/kg group. The proposed mechanism of Cd toxicity involves the binding of Cd to sulfhydryl groups on critical proteins, producing oxidative stress and the collapse of mitochondrial membrane potential$^{16}$. The remarkable increase in m-AST in the 6.3 mg/kg group may have been due to this mechanism. The liver has a very high capacity for metallothionein (MT) synthesis and for efficient trapping of Cd$^{17}$. Cd bound to MT within tissue is thought to be nontoxic; however, when a level of Cd exceeds the critical concentration, it becomes toxic$^{18}$. Therefore the hepatic injury was increased dramatically in the 6.3 mg/kg group. Cd induces the synthesis of MT and is then either stored in the liver as a Cd-MT complex or is transported via the blood to the kidney, where it accumulates in lysosomes$^{19}$. With increasing time after administration, there is an increase in renal Cd concentration, with lesions becoming apparent principally in the proximal convoluted tubules$^{20}$. This mechanism would affect decrease Cd excretion in the 2.1 mg/kg group. However, doses of CdN greater than 4.2 mg/kg may exceed the capacity of Cd accumulation. It has been reported that Cd-MT is filtered by the renal glomeruli and reabsorbed by proximal tubule lining cells, where it is catabolized, releasing Cd ions that cause renal damage$^{21}$. It has also been reported that extracellular Cd-MT is toxic to the kidney$^{21}$. The decrease in urine volume in the 6.3 mg/kg group indicated acute renal failure. The decrease in Ccr at doses...
of CdN greater than 4.2 mg/kg suggested acute glomerular dysfunction. The lack of change in urinary albumin did not indicate severe morphological glomerular injury. It has been reported that serum BUN and Cr were not markedly increased until the 3rd day after CdL (2.1 mg/kg Cd) injection. Thus, BUN and Cr levels elevate slowly. It is well known that NAG and β₂-microglobulin are indicators of proximal tubular injury. The increases in NAG/Cr, β₂-microglobulin and urinary glucose indicated proximal tubular injury in the 6.3 mg/kg group. The increase in urinary pH also indicated renal tubular injury. The decreases in BE, HCO₃⁻ within the reference range of anion gap, lactate and pyruvate indicated that renal tubular acidosis occurred in the 6.3 mg/kg group, leading to hyperventilation, decreased PCO₂ and increased PO₂. The changes in A-aDO₂ and O₂SaT indicated no respiratory disorder. Therefore, blood pH was kept within the reference range. The increases in FE₆⁷CO₂ and FE₆⁷O₂ within the reference range of relative densities were caused by disorder of reabsorption in the proximal tubules.

The secretion of K was severely affected by proximal tubular injury and severely decreased urine volume. Serum K was increased at doses greater than 4.2 mg/kg. It has been clinically reported that serum K greater than 5.5 mEq/L reflects hyperkalemia, and electrocardiogram is altered at 6.0 mEq/L. Severe hyperkalemia (between 6.1 and 7.0 mEq/L) may lead to atrioventricular dissociation, ventricular tachycardia, ventricular fibrillation or death, particularly if it develops acutely. Dose-related hyperkalemia is strongly associated with cardiac-derived lethal toxicity. Pathohistological renal tubular injury has been commonly caused by Cd exposure. However, there was no report of acute severe nephrotoxicity complicated with hyperkalemia in an in vivo study. Therefore, we suggest that CdN dominantly injures proximal tubules as the critical target as compared with other Cd compounds. Acute lethal toxicity could be related to hyperkalemia and would be one of the specific harmful effects of CdN.

Conclusion

The lethal effect of CdN would be promoted by exposure routes that cause rapid absorption of CdN into the body, such as inhalation, because of its solubility. Dramatic cytotoxic injury was caused by acute exposure of CdN that exceeded the critical capacity for detoxification, aggravating harmful systemic effects. Glomerular dysfunction and severe renal tubular injury rapidly caused abnormality of electrolytes and tubular acidosis. Hyperkalemia should be given special care in order not to worsen the prognosis.

References

17) Dudley RE, Gammal LM and Klaassen CD: Cadmium:


