Improvement in the Therapeutic Index of Cisplatin (NSC 119875) by Pharmacologically Induced Chloruresis in the Rat


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ABSTRACT

Low concentrations of chloride facilitate the conversion of cisplatin (CDDP) to cytotoxic species in aqueous solution. Low urine chloride concentrations increased CDDP-induced renal injury, while high urine chloride concentrations were protective. Sprague-Dawley rats drinking 1.2% sodium phosphate or 1.4% sodium bicarbonate and consuming low-chloride rat diet for 3 days excreted urine with a mean chloride concentration of 19.6 ± 11.3 mEq/liter. After drinking 1.0% sodium chloride with the same diet, rats which were treated with s.c. ammonium mercury (1 mg mercury per kg) on Day 3 of the experimental program developed a mean urine chloride concentration of 462 ± 172 mEq/liter. Weight, input, output, urine pH, and urine sodium concentration of the two groups did not differ. CDDP (5 mg/kg) i.p. produced greater renal injury in chloride-deprived rats than in control animals on normal diet and tap water and was nontoxic in the chloruresis group. In rats undergoing chloruresis, a dose of 10 mg CDDP per kg produced quantitatively similar renal injury to that produced by 5 mg CDDP per kg in control rats. Histological renal injury correlated with CDDP dose and was greatest in the distal tubules and collecting ducts of chloride-deprived rat kidneys. High-performance liquid chromatography fractionation of urine from catheterized rats demonstrated treatment-dependent speciation of CDDP. Urine from chloride-deprived rats contained 8.8 ± 2.1% of total platinum as non-CDDP species, while urine from the chloruresis group contained only 1.5 ± 0.6% as non-CDDP species (p < 0.01). The mammary carcinoma induced in female rats by i.v. N-nitrosomethylurea exhibited dose-related partial responses to a single dose of CDDP. There was a tumor induction rate of 100% in 120 rats with median latency of 87 days (range, 69 to 160 days) from the first of three monthly i.v. injections of 50 mg N-nitrosomethylurea per kg. The chloruresis regimen did not significantly affect the dose-response curve of CDDP in this model. Reduction of nephrotoxicity through altered urine platinum speciation, without loss of antitumor activity, showed that pharmacologically induced chloruresis improved the therapeutic index of CDDP.

INTRODUCTION

CDDP3 is the first platinum coordination complex to be used as an anticancer agent (39). The clinical utility of CDDP is well established (33), particularly in combination chemotherapy regimens for testicular carcinoma (1, 6, 7) and ovarian carcinoma (10, 42). Lesser activity is seen in squamous carcinoma of the cervix (43) or head and neck (48), bladder carcinoma (27, 41), and prostate carcinoma (26, 27). Evidence of minor activity is found in lymphomas (38) and pediatric tumors (16), but little or no activity is demonstrated in breast or colon carcinomas (18, 49). There is evidence that the therapeutic effect of CDDP exhibits a dose-response relationship (19). Measures which permit administration of higher doses of CDDP may convert modest antitumor effects into more impressive clinical responses.

Nephrotoxicity is a major dose-limiting side-effect of CDDP in humans (19, 24, 33, 39). Various experimental methods to reduce CDDP-induced nephrotoxicity are reported. An optimal protective regimen should specifically alter the renal microenvironment to inhibit the reactions of CDDP which produce nephrotoxicity and should not alter nonrenal reactions of CDDP. We have attempted to design such a regimen, based on established chemistry of CDDP.

The aquation reaction of CDDP (Chart 1) plays a dominant role in the chemistry of the drug in the physiological milieu (20). In chloride-free aqueous solution, CDDP (I) undergoes sequential loss of weakly bonded chloride ligands, with transient replacement by aquo ligands. The aquated forms (II, III, and V) are quite reactive with nucleophiles and can lose hydrogen ions to form the hydroxyl species (IV and VI). The latter are cytotoxic and can also form cytotoxic oxygen-bridged dimers and trimers (36). At equilibrium, the proportional distribution of total platinum between different complexes depends upon chloride concentration and, to a lesser extent, upon pH. The absolute concentration of each species depends upon the total platinum concentration as well. The influence of chloride ion on the activation of CDDP to cytotoxic complexes led us to explore the effects of alterations in urine chloride excretion on renal injury induced by CDDP in the rat, on urine speciation of CDDP, and on the therapeutic activity of CDDP in a previously unexplored rat tumor model.

Initially, we developed 2 treatment regimens which produced low and high urine chloride concentrations in rats. Physiological and histopathological parameters of nephrotoxicity showed greater injury caused by CDDP in chloride-deprived rats relative to control rats. The dominant site of microscopic injury was in the distal nephron, downstream from the tubular chloride retention mechanism. Relative to control rats, animals which excreted urine with high chloride concentration suffered less

nephrotoxicity. Urinary excretion of non-CDDP platinum species correlated with renal injury. The protective chloruresis regimen did not decrease the antitumor activity of CDDP in the NMU-induced rat mammary carcinoma system. Apparently, chloruresis improved the therapeutic index of CDDP through decreased renal activation of CDDP to cytotoxic complexes.

MATERIALS AND METHODS

Chemicals. CDDP was purchased from Bristol Laboratories, Syracuse, N. Y., as cis-diaminedichloroplatinum, in sterile amber vials containing CDDP (10 mg), mannitol (100 mg), and sodium chloride (90 mg). Vials were stored at 4°C and were reconstituted with 10 ml deionized water immediately prior to use. Chemically pure CDDP was purchased from Aldrich Chemical Co., Milwaukee, Wis., and atomic absorption platinum standard solution was purchased from Alfa Division of Ventron, Danvers, Mass. TM was purchased from Wyeth Laboratories, Inc., Philadelphia, Pa., as Thiomerin in clear sterile multiple-injection vials containing 125 mg TM (equivalent to 40 mg mercury) per ml. Injection solution was prepared by a 40:1 dilution of stock drug with 0.9% sodium chloride, and a dose of 1 mg mercury per kg was achieved by injecting 0.001 ml per g body weight. Low-chloride rat diet was purchased from Nutritional Biochemicals Division of ICN Life Sciences Group, Cleveland, Ohio, and control rat diet was Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.). All drinking solutions and the ammonium chloride injection solution were prepared from reagent-grade chemicals and deionized water. NMU was purchased from ICN Pharmaceuticals, Inc., Plainview, N. Y. An aqueous solution at a concentration of 10 mg/ml was made by wetting the drug powder with 3% acetic acid and then dissolving it in distilled water. A fresh solution was prepared and used immediately for each injection series.

Analytical and Histochemical Methods. Urine chloride concentrations were determined in triplicate by colormetric titration of silver electrolysate using a Buchler digital chlorideometer (Buchler Instrument Co., Fort Lee, N. J.). Urine sodium concentrations were determined by flame emission spectrophotometry using an IL-751 atomic absorption-emission spectrophotometer (Instrumentation Laboratories, Inc., Wilmington, Mass.). BUN and creatinine were measured with a single-channel Technicon Autoanalyzer II (Technicon Instruments Corp., Tarrytown, N. Y.). Kidneys were collected immediately after sacrifice with ether and were divided for fixation in buffered formalin. Specimens were prepared and stained with hematoxylin and eosin for light microscopy. Initial histopathological study was conducted by a pathologist (E.E.) who had not been informed of the experimental hypothesis. HPLC fractionation of platinum into CDDP and non-CDDP complexes was performed on a Varian Model 5020 liquid chromatograph with an automated 10-µl loop valve injector system. A guard column filled with Whatman ODS reverse-phase pellicular packing preceded a Varian MCH-10 analytical column (C18 reverse-phase bonded on 10-µm silica gel, 4 mm x 30 cm), maintained at 30°C with a column heater block. Ten-µl volumes of aqueous solutions were injected. The sample was eluted isocratically with 0.005 M tetrabutylammonium chloride in deionized water at a flow rate of 0.8 ml/min. Standards for retention times of CDDP and non-CDDP peaks were prepared by sonication of 1 mg CDDP per ml 0.9% NaCl solution or deionized water for 90 min, followed by aging at room temperature in plastic bottles for 12 hr to achieve speciation equilibrium. For standards, a Varian Varichrome variable wavelength detector with 8-µl flow cell monitored UV absorbance at 300 nm. Retention times were determined using a Varian CDS-401 chromatography data system. Retention times for non-CDDP and CDDP peaks were 2.6 and 4.1 min, respectively. Peaks were separated by 0.8 min at baseline. For samples with low platinum levels, fractions were collected every 30 sec with an LKB 2400 fraction collector. Fractions were diluted and analyzed in triplicate for platinum using an IL 751 atomic absorption spectrometer equipped with an IL 555 controlled temperature graphite furnace. The analysis program dried each 10-µl aliquot at 75–120°C (ramp) for 25 sec, pyrolized at 750–1600°C (ramp) for 45 sec, and atomized at 2700°C for 5 sec. Absorption was monitored at 265.9 nm, with deuterium lamp background correction. Diluted ACS standard H2PtCl6 solution yielded a linear standard curve (r2 = 0.9999) from 0.1 to 0.4 ppm platinum. Replicate analyses of CDDP at 10 ppm in 0.005 M tetrabutylammonium chloride yielded a relative S.D. of 3.2%. Replicate analyses of rat urine spiked with CDDP at 100 ppm yielded a relative S.D. of 2.2%.

Chloride Deprivation and Chloruresis Regimens. The overall experimental scheme is outlined in Chart 2. Male Sprague-Dawley origin rats weighing approximately 120 g were purchased from King Laboratories, Oregon, Wis., and were placed in individual stainless steel metabolism cages with free access to tap water by Richter tube and control rat diet. Control rats received this regimen for the entire experimental period. Animals were weighed daily, and urine was collected every 24 hr. After a 48-hr acclimatization period, experimental animals received one of 2 drinking fluid and diet regimens and were maintained on this intake for 72 hr. All animals were then returned to control diet and tap water for the duration of the study. Chloride deprivation was induced by a regimen of sodium phosphate in deionized water as ad libitum drinking fluid and low-chloride rat diet. Chloruresis was induced by a regimen of 1.0% sodium chloride drinking fluid and low-chloride rat diet. After 47 hr on the chloruresis regimen, maximal chloruresis was induced by 3 hourly s.c. injections of 250 mg ammonium chloride per kg as a 10% aqueous solution. Concomitant with the second injection, at Hr 48 of the experimental regimen, TM at a dose of 1.0 mg mercury per kg was injected s.c. Control, chloride-deprived, and chloruresis rats were all challenged at Hr 48 of their regimens with i.p. CDDP. Urine produced in the 4 hr following CDDP injection was collected and analyzed separately from the rest of the output for that day.

Regimen Modifications. Experiment 1 evaluated the time course of renal injury. Groups of 6 rats on chloruresis and chloride deprivation regimens were anesthetized with ether, bled by cardiac puncture, and sacrificed for renal histopathology each day for 5 days following i.p. injection of 5 mg CDDP per kg. CDDP was omitted for sets of control rats treated with each regimen. Experiment 2 substituted other anions for phosphate in the chloride deprivation regimen. Two groups of 6 rats received 1.0% sodium chloride solution, and 2 groups received 1.44% sodium bicarbonate, instead of 1.2% sodium phosphate drinking fluid. Groups on each regimen were treated with 5 or 10 mg CDDP per kg by i.p. injection and were bled by cardiac puncture 3 days later.

Urine Platinum Speciation. After 48 hr on the appropriate regimen, female rats were anesthetized with ketamine, and the perineal skin was infiltrated with lidocaine. A PE-50 polyethylene catheter with a small retention bead was introduced into the bladder via the urethra. A small skin incision exposed the urethra, and 2 sutures were used to ligate the urethra and secure the catheter. The distal catheter end was attached to the drop head of the fraction collector, and hourly urine fractions were collected. Chloruresis rats were given s.c. ammonium chloride and thimerin as noted above and were given additional s.c. 1.0% sodium chloride injections until urine chloride concentration exceeded 200 mEq/liter. Chloride-deprived rats were given s.c. 1.2% Na2HPO4 injections until urine chloride concentration was less than 50.
mEq/liter. When the appropriate chloride concentration was achieved, 5 mg of CDDP per kg were given i.p. Subsequent hourly urine collections were vortexed and filtered through 0.2-μm cellulose acetate filters (BAS microfilter system; Bioanalytical Systems, West Lafayette, Ind.) by centrifugation, and 10-μl aliquots were injected into the HPLC system within 10 min of the end of the collection period. When HPLC fractionation was performed on urine from the start and end of a 1-hr collection period, no differences in platinum speciation were found. An aliquot of each collection was diluted and analyzed for total platinum.

**Therapeutic Experiment.** Following the method of Gullino et al. (11), 120 fifty-day-old female Sprague-Dawley rats were given injections i.v. on Days 1, 29, and 57 with 50 mg of NMU per kg in aqueous solution. All rats were palpated weekly for tumors, lesions were measured with calipers, and the surface area of palpable masses was expressed as the product of the greatest diameter of the lesion and its greatest perpendicular diameter. When a group of 5 rats all bore at least one tumor with a cross-product of at least 1 sq cm, treatment of that group began. Each group of 5 rats was randomly assigned to one of 4 CDDP dose levels (1.25, 2.5, 5, and 10 mg/kg). Within each group, rats were randomly assigned to receive no treatment (one rat), CDDP alone (2 rats), or CDDP with the chloruresis regimen (2 rats).

Tumors were measured every 2 days for 20 days. A partial response was defined as a decrease in the sum of the cross-products of all measurable lesions to less than 50% of the cross-product sum on the day of treatment, for at least 2 consecutive observations, with no intercurrent new palpable lesions, in an animal which survived at least 6 days after CDDP administration. A complete response was defined as the disappearance of all palpable disease with the same observational constraints.

**RESULTS**

The regimens of Experiment 1 produced significant alterations in urine chloride concentrations. Chart 2 depicts the time course of means of urine sodium concentration and urine chloride concentration in this experiment. There were no statistically significant differences between the chloruresis and chloride deprivation regimens with regard to daily weight, fluid intake, volume of urine, or urine pH. All rats developed increased urine sodium concentration while on the chloride-loading and deprivation drinking fluids, reflecting the slightly hypertonic input. Equal concentrations of sodium were excreted on both regimens. Starting 2 to 3 days after CDDP administration, chloride-deprived animals lost body weight and drank less fluid, possibly reflecting general debilitation due to the drug. In the absence of CDDP, neither regimen produced azotemia, elevated serum creatinine, or microscopic renal abnormalities.

Experiment 1 also demonstrated significant differences between chloruresis and chloride deprivation regimens with regard to CDDP-induced renal injury, as shown in Chart 2. Mean BUN and serum creatinine values increased with time after CDDP treatment in groups of rats sacrificed each day for the 5 days following CDDP administration. These increases were statistically significant only in the chloride-deprived rats. Maximal azotemia appeared 3 or 4 days after CDDP administration, while creatinine maxima occurred 5 days after CDDP. Histological study of kidneys from these animals revealed minimal hydropic degeneration in proximal and distal tubular epithelial cells of rats treated with the chloruresis regimen (Figs. 1 and 2). In contrast, kidneys from chloride-deprived rats at the same time points (Figs. 3 and 4) showed more severe degeneration, which progressed to sloughing of tubular epithelium and hyaline cast formation at later time points. Injury to the distal tubular and collecting duct epithelium was more severe than was proximal tubular injury. The histological injury was time and dose related in all of our studies.

To test whether the increased CDDP-induced renal injury in chloride-deprived rats might be specifically related to high phosphate intake or excretion, we substituted 1.44% sodium bicarbonate for 1.2% sodium phosphate in the chloride deprivation program (Experiment 2). Both regimens produced urine chloride concentrations of 6 mEq/liter, with similar sodium, volume, and pH. At a CDDP dose of 5 mg/kg, rats given phosphate developed mean BUN of 111 ± 55 mg/dl, and rats given bicarbonate developed mean BUN of 241 ± 46 mg/dl.
Table 1

HPLC fractionation of urine collected in the first 2 hr after CDDP treatment (5 mg/kg) of rats undergoing chloruresis or chloride deprivation

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hr post-CDDP</th>
<th>pH</th>
<th>Urine [platinum] (ppm)</th>
<th>Volume (ml)</th>
<th>Total platinum (% of dose) as CDDP</th>
<th>μg platinum recovered as CDDP</th>
<th>μg platinum recovered as non-CDDP</th>
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<td>Chloruresis</td>
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<td></td>
<td></td>
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<tr>
<td>A</td>
<td>0–1</td>
<td>270</td>
<td>7.95</td>
<td>68</td>
<td>2.1</td>
<td>25.7</td>
<td>140 (98.8)</td>
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<td></td>
<td>1–2</td>
<td>239</td>
<td>6.71</td>
<td>37</td>
<td>2.4</td>
<td>16.1</td>
<td>88 (99.2)</td>
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<tr>
<td>B</td>
<td>0–1</td>
<td>293</td>
<td>5.68</td>
<td>64</td>
<td>1.7</td>
<td>15.3</td>
<td>107 (98.4)</td>
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<td></td>
<td>1–2</td>
<td>298</td>
<td>5.54</td>
<td>75</td>
<td>1.5</td>
<td>15.7</td>
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<td>3.7</td>
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<td>20</td>
<td>2.0</td>
<td>7.2</td>
<td>39 (97.5)</td>
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<tr>
<td>Mean</td>
<td></td>
<td>254</td>
<td>6.33</td>
<td></td>
<td>2.2</td>
<td>33.4</td>
<td>199 (98.5)</td>
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<tr>
<td>D</td>
<td>0–1</td>
<td>36</td>
<td>6.94</td>
<td>153</td>
<td>0.3</td>
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<td>1–2</td>
<td>26</td>
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<td>0.7</td>
<td>18.6</td>
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<tr>
<td>E</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0–1</td>
<td>10</td>
<td>5.93</td>
<td>90</td>
<td>1.0</td>
<td>10.2</td>
<td>83 (92.7)</td>
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<td>1–2</td>
<td>13</td>
<td>5.92</td>
<td>106</td>
<td>0.8</td>
<td>9.7</td>
<td>75 (88.2)</td>
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<tr>
<td>Total</td>
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<td>24</td>
<td>6.32</td>
<td></td>
<td>0.7</td>
<td>22.0</td>
<td>130 (91.2)</td>
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* Numbers in parentheses, percentage of total recovered.

b Mean of total 2-hr period.

c p < 0.01.

d p < 0.05.

Chart 3 displays results of HPLC fractionation of standards and rat urines. Chromatogram 3A resulted from injection of CDDP which had been dissolved in deionized water and allowed to equilibrate for 14 hr. The early peak is non-CDDP platinum-containing complexes. This peak disappeared if chloride was added to the solution. It was never present if CDDP was equilibrated similarly in a sodium chloride concentration of 160 mEq/liter. Chromatograms in Chart 3B are plots of analyzed platinum in 0.4-ml HPLC fractions, expressed as percentage of the total platinum recovered after injection of 10-μl aliquots of filtered rat urine. Data plotted as closed circles resulted from urine from a chloruresis rat (pH 5.92; platinum concentration, 19.9 ppm; and chloride concentration, 200 mEq/liter). Data plotted as open circles resulted from urine from a chloride-deprived rat (pH 5.92; platinum concentration, 106.2 ppm; and chloride concentration, 13 mEq/liter). Compared to the non-CDDP peak shown in Chart 3A, the non-CDDP peak shown in Chart 3B had a longer retention time. When sodium chloride was added to the urine to bring the chloride concentration to 200 mEq/liter, the urinary non-CDDP peak was not converted to CDDP.

The results of similar determinations in 6 rats are summarized in Table 1. With regard to the urine collections, there were significant differences between treatments in the chloride concentration and volume collected, and in the platinum concentration of urine collected in the second hr. Highly significant differences were seen in the percentage of recovered platinum found as CDDP versus non-CDDP species.

Both chloruresis and chloride deprivation regimens produced one different effect in the short-term, ketamine-anesthetized animals than in the conscious rats. The chloruresis regimen induced greater volume output than did the chloride deprivation regimen in anesthetized rats. Volume output was not different between regimens in the conscious animals. Urine pH and total platinum recovery were similar between regimens in both preparations.

The growth characteristics of the NMU-induced rat mammary carcinoma are depicted in Chart 4. Twenty-four untreated control rats were divided into groups which developed measurable tumors before (a) and after (b) the median date of occurrence. Each point is the mean of 12 animals.
The mean relative tumor cross-products of control rats are compared with similar means from all rats receiving 10 mg CDDP per kg in Chart 5. During the first week, a single dose of CDDP produced a significant decrease in the mean relative tumor size. After CDDP treatment, rats suffered significant transient weight loss relative to control animals. Statistical analysis of data corrected for animal weight demonstrated an independent effect of CDDP on tumor size.

Separate analysis of chloruresis and control groups showed no difference in tumor response to CDDP. Table 2 presents the number of partial responses produced by the 4 doses of CDDP and compares antitumor effect in control and chloruresis groups. The data were insufficient to establish accurate 50% lethal dose estimates, although there were fewer deaths at high doses in the chloruresis regimen group. Graphic analysis of these data by standard methods yielded the estimates of median effective dose and confidence interval shown in Table 2, which are not significantly different between treatment regimens.

DISCUSSION

A number of experimental studies address the problems of CDDP-induced nephrotoxicity. Sulfur-containing agents, such as penicillamine (13, 40) and thiourea (2), are used as platinum-binding agents but may interfere with therapeutic as well as toxic effects (29). Probenecid (37) and superoxide dismutase (25) show possible value in animals. The radioprotective agent WR-2721 [S-2-(3-aminopropylamino)ethylphosphorothioic acid] reduces CDDP nephrotoxicity in rats without shortening the dose-dependent delay in growth of transplanted rat mammary carcinomas (50). The greatest clinical benefit is derived from hydration, with or without pharmacological diuresis (3, 12, 47). Diuresis regimens are used in rodent models to decrease both acute (31, 45) and chronic (44) renal injury due to CDDP. One study also reports preservation of the therapeutic activity of the drug (32). Other workers are unable to demonstrate renal protection by these methods (21).

We wished to explore 3 experimental propositions: that CDDP-induced nephrotoxicity would be influenced by renal chloride excretion; that altered renal chloride excretion would change the chemical speciation of CDDP in urine; and that altered chloride excretion would have little effect on the antineoplastic activity of the drug. These propositions were deduced from the model of CDDP renal injury illustrated in Chart 6.

CDDP renal excretion occurs predominantly from the ultrafilterable fraction of total serum platinum (9, 30). This is thought to represent excretion of intact parent drug (5), at least in part. Clearance of ultrafilterable platinum equals or exceeds the creatinine clearance, implying that tubular secretion may occur (15). Thus, increasing concentration of platinum occurs in the luminal fluid as it passes down the nephron.

The thick ascending limb of Henle’s loop is the site of a specific active transport process for conservation of chloride. Under conditions of chloride deprivation, the tubular fluid:plasma chloride ratio may approach 0.001 (34). The chloride-dependent activation reaction of CDDP, shown in Chart 1, implies that intrarenal speciation of CDDP will ensue under these conditions. Important support for this point has been reported (23); chromatographically separable platinum species are found in human urine, and their ratio is related to the urine chloride content. The products of chloride-dependent activation of CDDP are reactive electrophiles and cytotoxic hydroxyl complexes and their dimers and trimers (36). We hypothesized that these species may produce renal injury to the tubular epithelial cells. The fraction of CDDP which is converted to cytotoxic species, and hence the degree of renal injury, may be altered by measures which alter intrarenal chloride handling.

If this model is correct, low urine chloride should enhance...
CDDP nephrotoxicity. By depriving rats of dietary chloride for 3 days, we induced selective chloride retention in rats. Such rats were more sensitive to CDDP-induced renal injury than were control animals. Extent of renal injury was independent of the anion which was substituted for chloride in the deprivation regimen.

The model also predicts that chloride deprivation should induce injury at sites distal to the major site of chloride removal from the luminal fluid. We found more histopathological evidence of severe injury in the distal tubular and collecting duct epithelium, consistent with the prediction of the model. Clinical studies have shown similar injury in humans (8). In contrast, proximal tubular injury predominates in some reported studies in the rat (17, 21, 31). In another study, Choie et al. (4) report both proximal and distal tubular epithelial injury, with maximal damage in the corticomedullary region. Possibly, the chloride-deprived rat model may mimic the clinical situation more closely than do other rat models.

Further implication of urinary chloride concentration as an important factor in CDDP-induced nephrotoxicity was found in the results of our chloruresis experiment. The combination of 1% sodium chloride drinking fluid, 750 mg ammonium chloride per kg s.c., and 1 mg (Hg) TM per kg s.c. was able to completely protect rats against nephrotoxic injury induced by 5 mg CDDP per kg i.p., as measured by biochemical and histopathological criteria. In addition, the chloruresis program reduced the severe injury caused by 10 mg CDDP per kg to approximately that caused by 5 mg CDDP per kg. This suggests that chloruresis may permit doubling of the CDDP dose which is clinically tolerable at present.

HPLC fractionation of urine platinum species was consistent with predictions of the model. The initial hydrolysis of CDDP in aqueous acid has a half-time of approximately 1.9 hr (22). Although temperature and other conditions can alter this rate, time between urine formation and HPLC analysis was not sufficient to account for the observed amounts of non-CDDP species as metabolites. The regimens achieved nearly identical urine pH, so it is unlikely that this factor caused differences in CDDP speciation between the groups. Although there was a difference in total platinum concentration between the groups, this factor should influence only the absolute concentration of non-CDDP species and not their percentile contribution to total urine platinum. We found significant differences in both the absolute and relative amounts of non-CDDP species, and these differences are probably attributable to the effects of chloride.

The nature of the non-CDDP species found in rat urine is under investigation. Since this material was not converted to CDDP by addition of chloride, it is probably not identical to the non-CDDP peak seen in chromatograms of CDDP after equilibration in deionized water. It may represent a complex which is formed in the reaction which produces renal injury. Chemical characterization of this material may improve understanding of the mechanisms which are involved in the toxic process.

The model also predicts that protection against CDDP-induced nephrotoxicity by chloruresis should not influence the antitumor activity of CDDP. This prediction was tested in the NMU-induced rat mammary carcinoma model as described. Analysis of data from untreated tumors arising early and late in the course of the study indicated that the growth rate of these lesions varies with the time required to achieve a given initial size. Although this finding was expected, it underscores the need for appropriate stratification and randomization as performed in this study.

CDDP produced a growth delay in the NMU-induced mammary carcinoma. No long-term survivors or complete responses were seen. The results were consistent with those achieved by others in the dimethylbenzanthracene-induced rat mammary carcinoma (46). The incidence of partial responses was clearly related to CDDP dose. Similar dose-response relationships are repeatedly observed in animal tumors, although clinical dose-response data are necessarily sparse. Chloruresis did not alter the median effective dose of CDDP in this system, supporting the model. Protection against CDDP-induced nephrotoxicity without abolition of therapeutic effect indicates that chloruresis improves the therapeutic index of CDDP in rats. Similar results have been achieved by others using mannitol in the Shay leukemia system in Sprague-Dawley rats (32) and using probenecid (37) or sodium thiosulfate (14) in the murine L1210 leukemia system.

If the toxic effects of CDDP are caused by chemical transformation to toxic species, several deductions can be made. Hydration regimens, with or without mannitol, probably protect the kidney by decreasing the absolute concentration of all platinum complexes. High urine chloride concentrations should confer an additional protective factor. The role of urine pH is less clear. Low pH will decrease the extent of aquation at equilibrium but will increase the concentration of the aquo species relative to the hydroxy species. Since the relative renal cytotoxicity of these species is not known, we cannot recommend a protective urine pH. Diuresis coupled with chloruresis is a desirable protective regimen in CDDP-induced renal injury.

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Fig. 1. Renal cortex from a rat on the chloruresis regimen of Experiment 1, sacrificed 3 days after CDDP (5 mg/kg). Although glomeruli appear normal, minimal degenerative changes are evidenced as cloudy swelling in epithelial cells of proximal convoluted tubules. H & E, × 220.

Fig. 2. Renal medulla from a rat on the chloruresis regimen of Experiment 1, sacrificed 3 days after CDDP (5 mg/kg). Distal tubular epithelial cells show perinuclear cytoplasmic clearing indicative of more severe hydropic degeneration than found in the proximal lesions of Fig. 1. H & E, × 220.

Fig. 3. Renal cortex from a rat on the chloride deprivation regimen of Experiment 1, sacrificed 3 days after CDDP (5 mg/kg). Proximal convoluted tubular epithelial cells show more severe injury than found in chloruresis animals. H & E, × 220.

Fig. 4. Renal corticomedullary junction from a rat on the chloride deprivation regimen of Experiment 1, sacrificed 3 days after CDDP (5 mg/kg). Proximal tubular cells from the pars recta show moderate injury, in contrast to severe degenerative changes and coagulative necrosis in the distal tubular and collecting duct epithelium. H & E, × 125.
Improvement in the Therapeutic Index of Cisplatin (NSC 119875) by Pharmacologically Induced Chloruresis in the Rat


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