Reduction of Cisplatin Nephrotoxicity by Procainamid: Does the Formation of a Cisplatin-Procainamid Complex Play a Role?1

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ABSTRACT

Procainamide protects mice bearing P388 leukemic cells against the toxicity of cisplatin without diminishing antitumor activity. The mechanism of action of procainamide protection was investigated both in vitro and in vivo. HPLC studies showed that procainamide forms a complex with cisplatin in vitro that has a UV spectrum similar to that of DPR, a triamine platinum complex that contains procaine as ligand. We report here the effect of the reaction product of cisplatin and procainamide on both cisplatin-induced DNA interstrand cross-links (ISCLs) and on the total DNA platination in vitro of isolated DNA. Total DNA platination in vitro of isolated DNA was increased by 113% (P < .01) and 17% (P < .05) after incubation times of 1.75 and 6 h, respectively, compared with products from the reaction of cisplatin with water. Furthermore, the reaction product of cisplatin and procainamide bound to DNA to a significantly greater extent than was cisplatin itself. ISCLs were decreased by 41% when this drug combination was incubated with DNA for 1.75 h, but no changes were observed after incubation for 6 h. We also examined the influence of the time interval between administration of cisplatin and procainamide on normal kidney injury, the renal distribution and urinary excretion of platinum, and the formation of cisplatin-DNA adducts in renal tissue of Sprague-Dawley rats after i.p. administration of 7.5 mg/kg cisplatin either with or without procainamide. The plasma concentrations of urea and creatinine and kidney histology demonstrated that procainamide provided effective protection in vivo in the rat when administered either simultaneously or at 0.5 and 1 h before or after cisplatin. The protection was accompanied by both higher renal levels of platinum and cisplatin-DNA adducts and by an increase in the formation of ISCLs. Moreover, a dose-dependent reduction of urinary excretion and concentration of platinum was also observed. We propose that procainamide, after accumulation in the kidney, may coordinate with cisplatin to form a less toxic DPR-like complex that renders rats less susceptible to cisplatin-induced toxicity.

In a previous paper (Esposito et al., 1996), we reported the results of studies on the use of procainamide for protection against cisplatin-induced nephrotoxicity in mice. This class I antiarrhythmic agent protected against death induced by lethal doses of cisplatin. The protective effect of procainamide against renal injury by cisplatin was demonstrated by both the plasma markers and kidney histology. Furthermore, the combination therapy of cisplatin and procainamide produced a significant increase in survival of mice bearing the P388 i.p. tumor, when the drugs were either simultaneously injected i.p. or by a different route of administration.

The initial study in mice did not focus on identification of the factors that influence the modulation of toxicity by procainamide. The rat is a good model to predict both qualitative and quantitative toxicity of cisplatin in humans (Graffner et al., 1979) and represents an animal species where the bioavailability of procainamide is similar to that reported in humans (Graffner et al., 1975; Schneck et al., 1978). Therefore, in this work the rat was used to evaluate the effect of varying the interval between the administration of cisplatin and procainamide on kidney toxicity and on the formation of cisplatin-DNA adducts.

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ABBREVIATIONS: NS, normal saline, 0.9% w/v NaCl solution; AAS, flameless atomic absorption; DPR, cis-diamminechloro-[2-(diethylamino)ethyl 4-aminobenzoate, N4]-chlorideplatinum(II) monohydrochloride monohydrate; ISCL, DNA-DNA interstrand cross-links; PUN, plasma urea nitrogen; TE, Tris-EDTA buffer; TLC, thin-layer chromatography; rt, retention time.
renal cisplatin-DNA adducts after combination therapy with either drug.

Direct chemical interaction between cisplatin and procainamide was suggested as one possible mechanism by which the combination treatment may protect normal tissue while retaining antitumor activity (Esposito et al., 1996). To test this hypothesis, we also investigated whether procainamide was able to react with cisplatin in vitro.

**Materials and Methods**

Cisplatin and procainamide hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). When the two drugs were administered to rats as single agents, cisplatin was dissolved in normal saline (NS; 0.9% w/v NaCl solution), whereas the modulating agent was diluted in distilled water to make a 1.25% solution. Because dissolving procainamide hydrochloride in NS increases the chloride anion concentration of the solution, when both drugs were administered together they were diluted in appropriate NaCl solutions to obtain a final [Cl−] equivalent to that of NS. Each drug sample was prepared fresh immediately before use. Acetonitrile and water of HPLC grade, Tris, acetic acid, methylene chloride, sodium hydroxide, isopropyl alcohol, all of analytical grade, were purchased from Merck (Darmstadt, Germany).

**In Vitro Experiments**

**Interaction Studies between Cisplatin and Procainamide.** To investigate the ability of procainamide to form a coordination complex with cisplatin, a study was performed in vitro. The time course of the reaction between procainamide and cisplatin was followed by HPLC. Cisplatin (0.5 mg/ml) was incubated with procainamide (5.4 mg/ml) in NS and maintained under stirring for 24 h in a water bath at 37°C. At definite time intervals, within the range 0 to 6 h, aliquots of this solution were withdrawn for HPLC analysis. Hydrolyzed cisplatin was obtained by incubating cisplatin (0.5 mg/ml) in deionized water in the dark at room temperature (23°C) for 48 h. The resulting procainamide solutions were maintained at 37°C in a constant temperature water bath and incubated in the dark from 0 to 6 h.

**Thin-Layer Chromatography (TLC) and HPLC Analyses.** TLC was carried out on RP8 precoated glass plates with fluorescent indicator (5 × 10 cm; Merck). The plates were activated by maintaining them at 110°C for 1 h. The ascending technique was used for development. The eluent system was 0.1 M tetraethylammonium chloride/methanol 20:10. The zones were located by UV irradiation at 254 nm and the presence of platinum was verified by dipping the plate into an ethereal solution of N,N-dimethyl-p-nitrosouanine, followed by heating at 120°C for 15 min (De Spiegeleer et al., 1984). A red color developed if platinum was present.

HPLC analyses were conducted using a Pump Series 4 (Perkin-Elmer, Norwalk, CT) equipped with a Rhodyne valve 7125-075 (20-μl loop; Cotati, CA) and a Diode Array Detector 1040 M Series II, managed by a ChemStation (Hewlett-Packard, Palo Alto, CA). The acquisition wavelength was 206 nm (bandwidth 4 nm).

The separation of cisplatin, procainamide, and unknown compound in samples was achieved by reverse-phase chromatography using a chemically bonded stationary phase, LiChrospher 100 CN (5 mm), packed into a LiChroCART (250 mm × 4 mm i.d.) cartridge. The column, operating at room temperature (20–24°C), was protected by a LiChroCART 25-4 precolumn containing LiChrosorb RP-8 (5 mm; Merck). The mobile phase consisted of 0.01 M dipotassium phosphate, pH 11.8) were added to 0.2-ml (20-μl) aliquots of DNA extracted from cells. Fluorescence was measured before and after heating at 90°C for 10 min (Perkin-Elmer LS-5B spectrophotofluorimeter; excitation wavelength, 525 nm; emission wavelength, 580 nm). The percentage of ISCL was determined by the formula (ft − fn)/(1 − fn) × 100, where ft and fn represent the fluorescence after denaturation divided by the fluorescence before denaturation of treated (ft) and control (fn) samples.

**Platinum Binding to BSA and DNA and DNA-DNA Interstrand Cross-Links (ISCLs) Formation.** A BSA solution was prepared in 0.01 M Tris-HCl buffer, pH 7.4. In all experiments, the final BSA concentration was 40 g/l, i.e., similar to that present in plasma. The DNA used in these experiments was extracted from P388 cells by the salting out technique (Gao et al., 1990) and resuspended in Tris-EDTA buffer (TE), pH 8. The DNA concentration in each experiment was 450 mg/ml, whereas its purity, expressed as the ratio between the absorbance at λ260 and λ280 nm, was on average 2.04 ± 0.07 (S.D.). Three different kinds of experiments were performed. In the first, 14.7 μM cisplatin plus 109 μM procainamide hydrochloride diluted in water were coincubated at 37°C for 3 h with BSA and for 1.75 and 6 h with DNA (coincubation; procainamide and cisplatin were present at a molar ratio of 7.4, i.e., similar to that used in vivo to examine the influence of timing and sequence of these compounds on normal tissue). Control samples were incubated with 14.7 μM cisplatin dissolved in a solution containing a chloride ion concentration similar to that of procainamide. After incubation, free platinum was separated from platinum-BSA by ultrafiltration in a Centrifree Micropartition Device (Amicon, Beverly, MA). DNA was separated by precipitation with one volume of isopropyl alcohol and resuspended in TE, pH 8. DNA yield was measured by absorbance at λ260 nm. Platinum was assayed by flameless atomic absorption (AAS; Pera and Harder, 1977).

In the second experiment, BSA and DNA were incubated with 14.7 μM cisplatin in NS at 37°C for 3 h, and 1.75 and 6 h, respectively. The resulting platinum-BSA complexes were then washed in 0.01 M Tris-HCl, pH 8, separated from free platinum by repeated centrifugation in Centriprep-30 Centrifugal Concentrators (Amicon), and finally resuspended in 0.01 M Tris-HCl, pH 7.4. DNA was precipitated, measured as described before, and resuspended in TE, pH 8. Platinum-BSA- and -DNA-containing solutions were then exposed to 109 μM procainamide diluted in NS at 37°C for 2 h. After this postincubation step, samples were processed and platinum was assayed as described above. The concentration of BSA at each step was measured according to the method of Lowry et al. (1951).

In the third experiment, the reaction product of cisplatin (0.5 mg/ml) and procainamide (3.4 mg/ml) was obtained by incubating the two drugs in water at 37°C for 24 h. BSA and DNA were then incubated at 37°C for 3 h, and 1.75 and 6 h, respectively, with the solution containing the reaction product of cisplatin with procainamide (the final concentration of platinum, calculated on the basis of the starting cisplatin concentration, was 14.7 μM). For comparison, a control cisplatin solution having a chloride anion concentration similar to that of procainamide was also incubated for 24 h at 37°C and investigated under the same experimental conditions. The percentage of ISCL was evaluated by the ethidium bromide fluorescence technique (Coluccia et al., 1995). DNA was resuspended in TE, and its yield and purity were estimated as before. Three milliliters of a solution containing ethidium bromide (0.5 mg/ml in 0.4 mM EDTA, 20 mM dipotassium phosphate, pH 11.8) were added to 0.2-ml (20-μl) aliquots of DNA extracted from cells. Fluorescence was measured before and after heating at 90°C for 10 min (Perkin-Elmer LS-5B spectrophotofluorimeter; excitation wavelength, 525 nm; emission wavelength, 580 nm). The percentage of ISCL was determined by the formula (ft − fn)/(1 − fn) × 100, where ft and fn represent the fluorescence after denaturation divided by the fluorescence before denaturation of treated (ft) and control (fn) samples.

**In Vivo Experiments**

**Animals.** Experiments were performed using male Sprague-Dawley rats (Charles River Laboratories, Calco, Italy) less than 1 year old with a body weight ranging from 400 to 550 g. Animals had free access to a commercial diet (4RF25; Italiana Mangimi, Settimo Milanese, Italy) and tap water and were kept in a temperature-controlled room. For urine sampling, animals were placed in metabolic cages 1 day before administration of the compounds.

**Timing and Sequence of Cisplatin in Relation to Procainamide.** Rats in groups of four to nine were injected i.p. with cisplatin either with or without procainamide. The dose for rats receiving cisplatin alone was 7.5 mg/kg cisplatin (LD50) in NS. Additional rats were treated with a single i.p. dose of procainamide (50 mg/kg) in water at 0 h (mixed with cisplatin) or at 0.5, 1, 2, and 4 h before or
after injection of cisplatin. One group was given i.p. only procainamide in water to serve as a control for the effects of the antiarrhythmic agent. The control group was injected i.p. with only NS.

**Renal Tissue Damage.** The influence of timing and sequence of cisplatin and procainamide administration on kidney toxicity was evaluated in normal rats at maximal cisplatin-induced toxicity (day 5 post-treatment). Renal function was examined by measurement of the concentrations of plasma urea nitrogen (PUN) and creatinine using the Beckman Liquid Stat test combination (Beckman Instruments Inc., Fullerton, CA).

**Histopathology.** Histopathologic changes in the kidney of rats sacrificed 5 days after injection of cisplatin either with or without procainamide were examined. The left kidney was removed from rats (n=5), the morphological studies were performed as described elsewhere (Esposito et al., 1990). The tissue sections were examined by light microscopy at 400× original magnification.

**Quantitation of Total Platinum, Platinum-DNA Binding, and ISCL in Kidney.** In an additional experiment, the effect of procainamide on the distribution of platinum as well as total DNA platination and ISCL formation in kidney was examined further. Twenty-four hours after treatment with cisplatin (7.5 mg/kg i.p.) either alone or combined with procainamide (100 mg/kg i.p.), kidneys from three to four normal rats per group were collected. The tissues were split into two parts. DNA was isolated from the first part of the tissues by the salting out technique (Gao et al., 1990). Briefly, tissue fragments (about 1-gram) were homogenized (Homogenizer Kinematica GmbH, Lucerne, Switzerland) in 15-ml vials containing 3 to 4 ml of PBS. Homogenized cells were then pelleted and washed twice in PBS by centrifugation at 750g for 10 min. Six milliliters of a lysis solution (10 mM Tris-HCl, pH 8; 2 mM EDTA, 400 mM NaCl), 240 μl of proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany), and 800 μl of 10% SDS (Bio-Rad Laboratories, Richmond, CA) were added. The solution was mixed gently and left overnight in a water bath at 37°C. After incubation, 2 ml of sodium acetate-saturated solution was added and mixed vigorously for 15 s. Vials were then centrifuged at 2500 rpm for 30 min, supernatants were recovered, and DNA was precipitated with one volume of isopropyl alcohol. Once isolated, DNA was dissolved overnight in TE solution (10 mM Tris-HCl, pH 8; 1 mM ethylenediamine tetraacetic acid, pH 8) at 50°C. DNA yield and purity were measured by absorbance at λ260 and λ280 nm [the purity of DNA was on average 2.02 ± 0.07 (S.D.)]; DNA was then processed either to determine the total DNA platination or the percentage of ISCL. For the analysis of total platination, DNA was digested in 14 M nitric acid and the residue was diluted in 10 mM nitric acid. Bound platinum was determined by AAS. The remaining part of the tissues was weighed and digested in 14 M nitric acid at 120°C. The residue was diluted in 10 mM nitric acid, and platinum content was evaluated by AAS. The percentage of ISCL in the kidney was evaluated by the ethidium bromide fluorescence technique as described elsewhere.

**Platinum Urinary Excretion.** A series of experiments was performed to compare urinary platinum excretion in normal rats treated with cisplatin (7.5 mg/kg i.p.) in the presence or absence of procainamide doses of 50 and 100 mg/kg. Urine samples from five to seven rats per group were collected at 2, 4, and 24 h after treatment. The concentration of platinum in urine was determined by AAS (Pera and Harder, 1977).

**Statistical Analysis.** Data were analyzed by both one-way ANOVA followed by a multiple comparison procedure (Newmann-Keuls test) and paired or unpaired Student’s t test. The level of significance was set at P < .05.

## Results

### In Vitro Experiments

**Kinetics of the Reaction between Cisplatin and Procainamide.** In the chromatogram of the solution of cisplatin incubated with procainamide in NS, a new signal [retention time (rt) = 3.6 min] was always present (Fig. 1A). The Purity Peak function of the HP Software indicated that the signal was due to a unique substance. To check this, the corresponding eluate fraction of a 24-h aliquot was collected and concentrated for TLC analysis. It gave a single spot (Rf = 0.38) that became red when the plate was dipped into an ethereal solution of N,N-dimethyl-p-nitroso-aniline, a reaction specific for platinum compounds. The platinum present in the fraction was also confirmed by AAS. Although no measurable hydrolysis of cisplatin in NS could be detected within 6 h, after incubation with procainamide we observed an increase in the concentration of platinum in urine was determined by AAS (Pera and Harder, 1977).

**Fig. 1.** A, representative HPLC chromatogram from the reaction mixture of cisplatin and procainamide hydrochloride in NS. 1: cisplatin; 2: unknown product; 3: procainamide hydrochloride. B, cisplatin loss in NS (●) and from reaction mixture of cisplatin and procainamide hydrochloride in NS (■). Vertical bars represent S.D. C, kinetics of the reactions of cisplatin and its hydrolysis forms with procainamide hydrochloride. The reactions of cisplatin (0.5 mg/ml) in NS (●) and hydrolysis forms of cisplatin (■) with procainamide hydrochloride (3.4 mg/ml) were followed by HPLC as described under Materials and Methods. Hydrolyzed cisplatin was obtained by incubating cisplatin (0.5 mg/ml) in deionized water in the dark at room temperature (23°C) for 48 h. The resulting procainamide solutions were maintained at 37°C in a constant temperature water bath and incubated in the dark from 0 to 6 h. Bars represent S.D.
in both the disappearance of parent drug (Fig. 1B) and time-dependent formation of the new platinum-containing product (Fig. 1C). Thus, it appears that the reaction with cisplatin occurs via a direct interaction. The UV spectrum of the unknown compound was very similar to that of *cis*-diammine-chloro-[2-(diethylamino)ethyl 4-aminobenzoate, N4-chlorideplatinum(II) monohydrochloride monohydrate (DPR), a triamine platinum complex containing procaine as ligand (Cafaggi et al., 1992) (Fig. 2). In the HPLC and TLC system used, DPR behaved quite similarly to the unknown compound, with a peak at *t* = 4.0 min and a spot at *R* = 0.23. Because the products formed in vivo from cisplatin after the hydrolysis of the chloride atoms with cellular components are presumably the active form of this drug, the reaction of procainamide with the hydrolysis products of cisplatin was also determined. Under these circumstances, the same reaction product as that found after incubation in NS was obtained, although it formed more readily (Fig. 1C).

**BSA and DNA Binding Studies.** The effect of procainamide on the binding of platinum to BSA and DNA, as well as on the formation of ISCLs in vitro, can be seen in Table 1. Compared with the controls, no effects in BSA-bound platinum and DNA platination or in percentage of ISCL were observed after coincubation of BSA and DNA with cisplatin and procainamide for 3 and 6 h, respectively. A 2-h exposure to the modulating agent produced only a small reversal of cisplatin-DNA adducts formed by the reaction of DNA with cisplatin for 6 h, whereas alterations of percentage of ISCL were not observed (postincubation, Table 1). The reversal of platinum from BSA did not occur at all with procainamide. Notably, exposure of BSA and DNA to the reaction product of cisplatin with procainamide resulted in a 23% (*P* < .01) increase of BSA-bound platinum, and in 113% (*P* < .01) and 17% (*P* < .05) increases of DNA platination after incubation times of 1.75 and 6 h, respectively. After incubation with DNA for 1.75 h, this combination led to significantly lower (−41%, *P* < .01) levels of ISCL compared with the controls, whereas when DNA was incubated with the reaction product of cisplatin and procainamide for 6 h, no significant differences were observed (Table 1).

**In Vivo Experiments**

**Influence of Sequence and Timing of Cisplatin and Procainamide on Kidney Function.** Five days after the treatment with 7.5 mg/kg cisplatin, rats showed an evident and significant increase of PUN and creatinine plasma concentrations (*P* < .01) compared with animals receiving NS alone. PUN and creatinine levels at death 5 days after treatment with only procainamide were comparable to those observed in controls. Reductions in PUN and creatinine concentrations (*P* < .01) were noted when procainamide was administered at times ranging from 1 h before cisplatin to 1 h thereafter, as well as after giving the two drugs mixed together (Table 2). Procainamide administration between 2 and 4 h before or after cisplatin, however, failed to demonstrate a significant reduction either in PUN or creatinine levels.

**Histopathology.** Histological examination of renal slices from rats treated with cisplatin alone showed different alterations, including a shorter tubular epithelium, a loss of striated edge in proximal tubules, a discontinuity of tubular wall, and an increase of intertubular connective tissue (Fig. 3A). The pyramids and the renal corpuscle presented a normal morphology. Slices derived from rats treated with procainamide alone showed only a moderate increase of intertubular connective tissue (Fig. 3B). Tubules of the kidneys of rats treated with cisplatin plus procainamide (Fig. 3C) showed considerably less degeneration and less cell loss of the tubular epithelium at day 5 post-treatment than those of rats treated with 7.5 mg/kg cisplatin alone. The quantitative evaluation of renal damage showed that the combination therapy of cisplatin and procainamide reduced the percentage of proximal tubules showing abnormal morphology to only 13%, compared with treatment with either cisplatin alone (94%) or procainamide (22%) (Fig. 4).

**Effect of Cisplatin with or without Procainamide on Total Platinum, DNA Platination and ISCL in Kidneys.** The concentrations of platinum in kidney and in its extracted DNA 24 h after cisplatin administration either with or without procainamide are shown in Table 3. Statistically significant higher tissue concentrations of platinum were observed in the kidney of rats receiving cisplatin together with procainamide, compared with animals treated with cisplatin alone (*P* < .05). In rats receiving the combined treatment of cisplatin and procainamide, the total platination of DNA in kidney was increased by an average of 78%, compared with animals treated with only cisplatin. The combination of cisplatin and procainamide also showed a 1.6-fold increase in formation of ISCL in renal tissue (Table 3). The renal platinum concentrations were also increased when the antiarrhythmic drug was administered 0.5 h before or after a single injection of cisplatin (Fig. 5).

**Influence of Procainamide on the Urinary Excretion of Platinum.** The data in Fig. 6 suggest a dose-dependent effect of procainamide on the urinary excretion of platinum. Over the entire 24-h period, the reduction in urinary platinum excretion by procainamide ranged from 40% (*P* < .05) to 95% (*P* < .01) at doses of 50 and 100 mg/kg, respectively (Fig. 6A). This effect was associated with a statistically significant reduction in the urinary concentration of platinum (Fig. 6B). At each collection period, urine volumes from rats treated with cisplatin together with 50 mg/kg procainamide were practically equal to those from animals receiving only cisplatin. Although a trend toward lower urine volumes was noted in animals receiving a procainamide dose of 100 mg/kg, the effect did not reach statistical significance (Fig. 6C).

**Discussion**

The mechanism of the selective protection against cisplatin-induced nephrotoxicity by procainamide is presently unknown. The primary aim of this study was to gain an
TABLE 1

Binding of platinum to BSA and DNA in vitro and the percentage of ISCL after incubation of cisplatin with or without procainamide

Co-incubation: cisplatin (14.7 μM) was incubated alone or together with 109 μM procainamide. Post-incubation: after preincubation with cisplatin (14.7 μM) for 3 h with BSA, and 1.75 and 6 h with DNA, BSA and DNA were separated, washed, and incubated either with procainamide (109 μM) or normal saline at 37°C for 2 h (see Materials and Methods). Reaction products, after incubation at 37°C for 24 h, of cisplatin (0.5 mg/ml) with procainamide (3.4 mg/ml) or a saline solution containing a chloride anion concentration equal to that of 0.34% procainamide aqueous solution (the final concentrations of cisplatin and procainamide were 14.7 and 109 μM, respectively).

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>Coincubation</th>
<th>Postincubation</th>
<th>Reaction Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cisplatin</td>
<td>Cisplatin + Procainamide</td>
<td>Cisplatin</td>
</tr>
<tr>
<td>DNA-bound platinum (ng of platinum/ mg of DNA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>1.6 ± 0.4a</td>
<td>1.8 ± 0.5</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>3.9 ± 0.5</td>
<td>3.8 ± 0.7</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Percentage of ISCL</td>
<td>1.75</td>
<td>34.8 ± 5.7</td>
<td>20.6 ± 5.9</td>
</tr>
<tr>
<td>6</td>
<td>56.5 ± 7.9</td>
<td>56.2 ± 8</td>
<td>59.2 ± 2.9</td>
</tr>
<tr>
<td>BSA-bound platinum (ng of platinum/ mg of BSA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34.1 ± 13.5</td>
<td>33.9 ± 15.1</td>
<td>31.4 ± 12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46.8 ± 11.6</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.D. of four to nine separate experiments.

** Significantly different (P < 0.01) from their own cisplatin controls (paired Student’s t test).

*** Significantly different (P < 0.05) from their own cisplatin controls (paired Student’s t test).

TABLE 2

Influence of the sequence and timing of administration of cisplatin and procainamide on PUN and creatinine values in rats

PUN, creatinine, and body weight were determined at day 5 after the treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>PUN mg/dl</th>
<th>Creatinine</th>
<th>mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>19 ± 1</td>
<td>0.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>287 ± 69</td>
<td>7.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Procainamide</td>
<td>20 ± 0.7</td>
<td>0.5 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Cisplatin + Procainamide</td>
<td>306 ± 16</td>
<td>7.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>-4 h</td>
<td>321 ± 17</td>
<td>6.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>-1 h</td>
<td>79 ± 5a</td>
<td>2.2 ± 0.4a</td>
<td></td>
</tr>
<tr>
<td>-0.5 h</td>
<td>56 ± 12a</td>
<td>1.7 ± 0.4a</td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>93 ± 56b</td>
<td>2.9 ± 2.2b</td>
<td></td>
</tr>
<tr>
<td>+0.5 h</td>
<td>60 ± 35a</td>
<td>1.6 ± 0.9a</td>
<td></td>
</tr>
<tr>
<td>+1 h</td>
<td>107 ± 59a</td>
<td>2.8 ± 1.5a</td>
<td></td>
</tr>
<tr>
<td>+2 h</td>
<td>288 ± 45</td>
<td>5.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>+4 h</td>
<td>318 ± 19</td>
<td>6.9 ± 2.6</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.01, compared with cisplatin alone.

** Not significant when compared with -1, -0.5, +0.5, and +1 h procainamide-treated groups.

Insight into the possible mechanism(s) of this protective effect. The in vitro results presented here have demonstrated that the antiarrhythmic drug procainamide reacts with cisplatin and its hydrolysis products in vitro to generate a new platinum compound. The data from the experiments in vivo clearly indicate that procainamide selectively reduces cisplatin-induced nephrotoxicity in the rat model, either when administered simultaneously with cisplatin or when given 0.5 and 1 h before or after cisplatin. Renal histopathology showed that although single agent treatment with cisplatin or procainamide induced structural alterations of the cortical zone of the kidneys, when the compounds were given together the renal damage decreased or even disappeared. Similar findings were obtained in the experiments in mice (Esposito et al., 1996).

The observation that procainamide also protects against cisplatin-induced renal injury when given as a rescue agent (i.e., at 0.5- and 1-h intervals after cisplatin) suggests that the time of administration is less critical for the antiarrhythmic drug than for other protectors (Gandara et al., 1990). Most of the damage to kidneys of rodents has been reported to appear within 0.5 to 1 h after the administration of a toxic dose of cisplatin (Borch and Pleasant, 1979). This interval corresponds to maximal renal accumulation and to a higher urine concentration of platinum. Procainamide has a rapid distribution phase in the rat (t1/2 = 10 min) and the t1/2 for the β-phase of plasma elimination is about 55 min. Effective plasma concentrations of procainamide (i.e., 4 mg/ml) are achieved up to 1 h after a single i.v. dose of 50 mg/kg with extensive tissue distribution, particularly in kidney and liver (Schneck et al., 1978). The failure of procainamide to either protect or rescue at 2 and 4 h is presumably due to cisplatin-induced alterations that are either inadequate to prevent the toxic effects of cisplatin in kidneys, when the antiarrhythmic agent is given at these intervals before cisplatin, or to platinum-induced irreversible damage having already occurred within 2 h after the administration of cisplatin.

It has been reported that tissues showing clear cisplatin-induced histological alterations also show moderate to high levels of cisplatin-DNA interaction products (Terheggen et al., 1987). Once inside the cell, cisplatin is converted to a hydrated Pt(II) coordination complex that forms ISCL and intradna cross-links. Although the role of each type of cross-link in the effects of cisplatin remains a subject of debate, cisplatin-induced ISCLs have been found to positively correlate with the cytotoxicity of the drug (Roberts et al., 1986; Yoshida et al., 1994). Contrary to what might have been expected, procainamide treatment was accompanied by a decrease in both renal excretion and urinary concentration of platinum as well as by an increase in total DNA platination and ISCL in the kidney. Fluid output (as urinary volume) was not significantly affected by procainamide treatment. The results also show that the platinum concentrations in the kidneys were elevated either when cisplatin and procainamide were combined or when the antiarrhythmic agent was administered 0.5 h before or after cisplatin (Fig 5), although these schedules of administration were effective in reducing cisplatin-induced nephrotoxicity. These findings suggest that the protective action of procainamide could not depend on the prevention of cisplatin entry into the cells. The combination of rather high residual levels of renal platinum and reduced nephrotoxicity found in rats treated with procainamide is somewhat surprising. However, the lack of a correlation between platinum levels in the kidney and renal damage in rats also has been found for other modulating agents (Natrochin et al., 1989; Basinger et al., 1990). It is possible that the reduction of nephrotoxicity provided by
Procainamide involves a specific interaction of the antiarrhythmic agent with cisplatin or its aquated forms, which results in enhanced levels of a less toxic platinum compound.

Cisplatin is filtered and actively secreted in the renal tubules (Reece et al., 1985). There is evidence that renal secretion of cisplatin or its metabolites occurs, at least in part, via an organic cation transport system (Daley-Yates and McBrien, 1982; Williams and Hottendorf, 1985). With this system, procainamide is actively secreted through the kidney (Bauman, 1988). Both cisplatin and procainamide are organic bases and competition for tubular secretion of basic drugs has been reported (Somogyi et al., 1983). Previous data in dogs showed that the renal clearance of cisplatin decreased significantly during and shortly after infusion of drugs sharing the cation transport system, whereas no differences in urine flow or in serum protein binding of platinum were observed (Klein et al., 1991). These findings along with those from our experiments in rats point toward the notion that a combined treatment with organic cations and cisplatin may decrease the urinary excretion of platinum.

The pharmacological basis for renal damage by cisplatin probably depends on its highly reactive, positively charged, hydrated metabolites, which may be transported by the organic cation system (Bird et al., 1984; Klein et al., 1991) and bind to sulfhydryl groups on the renal tubule at high plasma and urine platinum concentrations (Borsch and Pleasant, 1979). Transport of active species of platinum from cell to tubular lumen may be reduced by procainamide. Alternatively, procainamide may alter nontransport events, such as cellular distribution of cisplatin to crucial subcellular organelles such as mitochondria and/or factors regulating cell death mechanisms (Zhang and Lindup, 1993, 1994).

Our studies in vitro made it apparent that procainamide is not able to alter the affinity of platinum for BSA and DNA. Therefore, the higher binding of platinum to renal tissue and the greater DNA platination in the kidney that were observed in the presence of the modulating agent in rats cannot be explained by a simple procainamide-induced effect on the binding of cisplatin to either plasma proteins or DNA when the two drugs are given simultaneously. In this context, the reaction kinetics of cisplatin and procainamide were interesting. The data indicated the ability of procainamide to form a coordination complex with cisplatin. No attempts have been made to characterize this product, but it behaved sim-
ilarly to DPR (Fig. 2), a platinum coordination complex with procaine previously characterized by us (Cafaggi et al., 1992). Given that procainamide contains the same primary aromatic amino group that is responsible for the complexation between cisplatin and procaine, it seems reasonable to expect a parallel complexation between cisplatin and the antiarrhythmic agent. DPR induces fewer nephrotoxic (Cafaggi et al., 1992; Zhang et al., 1996) and neurotoxic (Mandys et al., 1998) effects than cisplatin. Moreover, this platinum compound has a greater ability to platinate DNA in vitro than cisplatin (Viale et al., 1995). As has been suggested for DPR (Viale et al., 1996) and for other monofunctional agents (Payet et al., 1993), this new cisplatin-procainamide compound could form unstable monofunctional adducts with DNA that, owing to the low intracellular chloride concentration and/or the structure of the DNA (i.e., base sequence), could generate bifunctional adducts by a two-step reaction. This notion is in keeping with our studies in vitro demonstrating that the reaction product of cisplatin and procainamide binds to DNA and BSA to a greater extent than either cisplatin itself or the products from reaction of cisplatin with water. Furthermore, the reaction products derived from the combination of cisplatin and procainamide can form interstrand cross-links on DNA, whose formation seems to occur in vitro at a slower rate than that occurring after incubation of DNA with the reaction products of cisplatin and
water (Table 1). Although care must be taken when extrapolating from in vitro results, if this interaction occurred in vivo, then it would explain the higher tissue platinum levels, as well as the higher amounts of total platinum-DNA binding found in kidneys of rats in the presence of procainamide. It is more difficult to explain the higher percentage of ISCL found in kidneys of rats treated with cisplatin and procainamide compared with animals that received cisplatin alone. In vivo a greater transformation of monofunctional to bifunctional adducts, to produce values higher than those found in cisplatin-treated rats, cannot be dismissed.

Experiments in vivo demonstrated that procainamide also protected against cisplatin-induced kidney damage when administered up to 1 h after cisplatin and that the renal protection afforded by the antiarrhythmic agent was accompanied by an increased platinum accumulation in renal tissue. The results of experiments in vitro, demonstrating that procainamide did not reverse BSA-bound platinum and was hardly able to reverse the already formed platinum-DNA adducts, suggest that the reversal of platinum-induced damage by procainamide should not be an important mechanism of protection. Overall, our in vitro and in vivo results support the hypothesis that the modulating agent can offer protection against cisplatin-induced toxicity by preventing cellular damage. In rats, procainamide is extensively distributed in tissues, particularly in kidney, whereas cisplatin is cleared quickly from the circulation by renal extraction and protein binding. Because the events responsible for the toxicity of cisplatin occur soon after its administration, it is expected that the prevention of damage to the kidney caused by cisplatin may be highly dependent on the ability of this tissue to efficiently accumulate procainamide. Based on the capability of procainamide to react with cisplatin and its hydrolysis products, we propose that the modulating agent, after accumulation in the kidney, may react with cisplatin to form a less toxic DPR-like coordination complex, rendering rats less susceptible to cisplatin-induced toxicity.

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References

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