Anti-Cancer Drugs, 4, pp. 149–162

Cisplatin and carboplatin induced changes in the neurohypophysis and parathyroid, and their role in nephrotoxicity

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Cytochemical and ultrastructural studies in Wistar rats [Crl: (WI)BR] show that cisplatin treatment (5–9 mg/kg) induces a release of neurosecretory granules from the neurohypophysis with a corresponding decrease in the urine output in a time and dose dependent fashion. Cisplatin induces low blood calcium and phosphate levels with a corresponding increase in the dark cells of the parathyroid gland. Injections of calcium before and during the treatment of cisplatin are effective in combating hypocalcemia, nephrotoxicity and gastro-intestinal toxicity due to cisplatin. Carboplatin, a less nephrotoxic agent, does not demonstrate any of these changes.

Key words: Carboplatin, cisplatin, nephrotoxicity, neurohypophysis, parathyroid, vasopressin.

Introduction

Cisplatin is one of the most valuable antineoplastic drugs[1,2] currently in use for the treatment of ovarian and testicular cancers.[3–5] When used in combination therapy with other antitumor agents, it has proven to be effective against cancers of the head, neck, bladder, prostate, lung and cervix.[3,4,6] It does, however, produce nephrotoxicity, nausea and vomiting, which have been the major dose-limiting factors in its use.[7,8] Cisplatin kills differentiated spermatogonia[8,9] in mice and monkeys, and is embryotoxic in rats and mice.[10,11]

In clinical treatment, the gastro-intestinal toxicity manifests itself as nausea and vomiting within hours of treatment, and delayed emesis is sometimes pronounced 48–72 h post-treatment in 60–80% of patients.[12,13] In laboratory investigations involving rats and mice the gastro-intestinal toxicity is characterized by bloated stomachs, diarrhea and paralysis of intestinal peristalsis.[14] The incidence of stomach bloating has been found to parallel the emesis associated with the clinical use of cisplatin and other chemotherapeutic agents.[15] The mechanism of cisplatin antitumor action is still unclear.[16,17] The primary mechanism of its action has been proposed to be through cross-linking with DNA.[18–21] However, it has also been shown to inhibit karyokinesis and cytokinesis through depolymerization of microfilaments due to cytosolic calcium changes.[22,23] Cisplatin has been shown to effect various transport enzymes,[24–26] and induce hypocalcemia and hypomagnesemia.[27] Cisplatin also induces morphological changes in the kidney and diminished urine output depending upon the species or the strain of animal being used.[28]

Carboplatin, a second generation analog of cisplatin, however, is less nephrotoxic, does not show any gastro-intestinal toxicity and is diuretic in some animals.[28,29] Cisplatin induces diuresis in Long-Evans rats whereas carboplatin is antidiuretic and more toxic in the same strain.[28] Thus, the two drugs influence renal function differently in the same strain. Induction of diuresis has been used as a means to ameliorate the nephrotoxicity of cisplatin; apparently this prevents cisplatin accumulation in the kidney.[30] Since water excretion is influenced in part by the antidiuretic hormone (VSP) and blood calcium level is influenced by the secretions of the parathyroid gland, it was essential to determine if cisplatin and carboplatin induce any changes in the posterior pituitary and the parathyroid glands. The present study is an effort to determine the effect of these two drugs on

This work was supported in part by BRSG grant 2-507-RR07049-15 from the National Institute of Health.

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morphological and cytochemical changes in the neurohypophysis, and correlate these to any diuretic or antidiuretic influence on the kidney or any hormonal changes in the parathyroid gland that may influence the calcium levels in the body.

Materials and methods

Animals

Laboratory-bred male Wistar rats [Crl: (WI)BR] (Charles River Breeding Lab, Portage, MI) weighing 200–300 g and approximately 3 months of age were used in these experiments. Animals were divided into groups of 20 and were treated with 5, 7 or 9 mg cisplatin/kg in 0.85% saline or carboplatin (50 mg/kg) dissolved in 5% glucose on day 1 as a single intraperitoneal injection (see also Table 1). Control animals received saline or glucose only. The animals were housed in metabolic cages and provided with Wayne Laboratory animal feed (Allied Mills, Chicago, IL) and tap water *ad libitum*.

Table 1. Experimental design

Group	Treatment	Animals killed at days after treatment				
		3	5	10	45	60
I a	cisplatin (5 mg/kg)	20	20	20	10	10
b	cisplatin (7 mg/kg)	20	20	20	10	10
c	cisplatin (9 mg/kg)	20	20	20	20	20
II a	cisplatin (5 mg/kg) + calcium (0.5 ml 10% calcium gluconate)	20	20	20	20	20
b	cisplatin (7 mg/kg) + calcium (0.5 ml 10% calcium gluconate)	20	20	10	10	10
c	cisplatin (9 mg/kg) + calcium (0.5 ml 10% calcium gluconate)	20	20	10	10	10
III	normal saline (0.85% NaCl)	20	20	20	20	20
IV	normal + saline + calcium (0.5 ml 10% calcium gluconate)	20	20	0	0	0
V	carboplatin (50 mg/kg)	20	20	0	0	0
VI	normal + glucose (5%)	20	20	0	0	0
VII	normal-water deprived	20	20	0	0	0

Urine output and water intake were monitored for each rat throughout the experiment. Treated and control animals were given daily intravenous injections of calcium (0.5 ml of 10% calcium gluconate or 1.1 ml of 1.3% $CaCl_2$) at 08:00 h on the day of cisplatin/carboplatin treatment and each day thereafter up to the day of euthanasia. Animals were euthanatized by decapitation on days 3, 5, 10, 45 and 60 post-treatment. Free-flowing trunk blood from decapitated animals was collected and centrifuged at 500 *g* for 10 min. Plasma was stored frozen at −20°C until assayed. Calcium measurements were done on the urine and plasma using atomic absorption spectroscopy. To assess the effect of dehydration on the neurohypophysis a group of 20 animals was deprived of water for up to 5 days. These animals were euthanatized on day 1 or 5 and the pituitaries were processed for light or electron microscopy. The stomachs from various drug treatments or controls were removed and photographed for bloating or non-bloating. The weights of these stomachs along with their contents were also recorded (see Table 2). After weighing, the stomachs were checked for ulceration by opening through an incision along the basal surface. Photographic records were made of any ulcerations.

Tissue handling procedures

The pituitary and parathyroid gland tissues were fixed in Bouin's fluid for 4 h and processed in a routine manner for paraffin sectioning.[31] The activity of the parathyroid gland (dark cells versus light cells) was measured by computer-assisted image analysis using a General Imaging (Gainesville, FL) analysis system. Sections (1 μm thick) of the parathyroid gland were stained with 1% solution of methylene blue in 1% borax for 60 s at 60°C and viewed under a Leitz Dialux 20 microscope equipped with a Dage MT1 67M video camera. The image was digitized with a FG100 frame grabber (Imaging Technology, Woburn, MA). Intensely staining areas after methylene blue were automatically highlighted and the number of pixels in such areas was counted. The total area of the gland in pixels was determined and the percentage of dark cells calculated. Images were processed and analyzed using software from Micro Science (Federal Way, WA). Three sections from each gland separated by a distance of no less than 50 μm were used in this analysis. Five random animals from each group were utilized for the above analysis.

Table 2. Stomach distension after a single intraperitoneal injection of cisplatin in male Wistar rats

Dosage of cisplatin[a] (mg/kg)	Mean stomach weight (g/100 g body weight) plus contents on day post-treatment[b]				
	+1	+2	+3	+4	+5
0 (Saline alone)	1.5 ± 1.1	1.3 ± 1	1.4 ± 1.5	1.4 ± 0.9	1.5 ± 1.1
6	2.9 ± 1.2[d]	4.7 ± 1.3[d]	4.6 ± 1.2[d]	5.2 ± 2[d]	6.0 ± 1.2[d]
9	3.2 ± 1.7[d]	4.1 ± 0.7[d]	5.3 ± 0.9[d]	7.7 ± 0.8[d]	8.2 ± 0.7[d]
9 + calcium[c]	2.1 ± 1.1	2.2 ± 0.8	2.3 ± 0.9	2.2 ± 1.1	2.4 ± 0.9

[a] Given intraperitoneally as 0.15 M NaCl on day 0.
[b] $n = 10$/group.
[c] Calcium (5 mg) was administered daily as 1.1 ml of 1.3% $CaCl_2$ (w/v) through the tail vein.
[d] Significantly different from the saline treatment alone ($p < 0.05$).

Histochemistry

Sections (7 μm) were stained with the Periodic acid–Schiff (PAS) technique before and after salivary amylase digestion, and were counter stained with either Orange G or Ehrlich hematoxylin. Chromalum hematoxylin/eosin was used to stain the adjacent sections for the demonstration of neural hormones and pituicyte morphology.

Effect of cisplatin on frozen kidney sections for alkaline phosphatase

In order to study the direct effects of cisplatin on alkaline phosphatase, fresh frozen sections (7 μm) of kidney from normal rats were incubated for 1–4 h in cisplatin (7–9 mg/100 ml saline) both before and after 15 min of formaldehyde (4% in cacodylate buffer, pH 7.2) fixation. Sections were incubated for alkaline phosphatase activity as described below.

Alkaline phosphatase

Alkaline phosphatase activity was visualized by incubating thick frozen sections (7 μm) of the kidneys from treated and control animals in medium containing 0.2 M Tris–maleate buffer (pH 8.2), sodium β-glycerophosphate (1.2%), 1% lead nitrate, 0.2 mM magnesium chloride and distilled water for 45 min at 37°C according to the method of Hugon and Borgers.[32] The control incubation medium contained 50 mM L-phenylalanine or 0.05 mM levamisole.[33] The staining intensities were measured by computer-assisted image analysis as described earlier.

For the immunocytochemical localization of VSP, primary antisera, rabbit anti-Arg[8] VSP (Biomedia, Forest City, CA) was diluted to a final working solution of 1:50. Secondary antisera used was goat anti-rabbit IgG, conjugated with alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) in a dilution of 1:100. Sections of formaldehyde fixed pituitaries were incubated in primary antisera for 30 min at room temperature. After proper rinses, sections were incubated in secondary antisera for 30 min. Binding of the labeled antibody was detected by the histochemical demonstration of alkaline phosphatase.[32] Endogenous alkaline phosphatase activity was inhibited by prior incubation in 0.05 mM levamisole.[33] Controls included omitting either the primary or secondary antisera. Frozen sections of kidney tissue served as positive controls for alkaline phosphatase activity.[34]

Electron microscopy

For electron microscopy, pituitary and parathyroid tissues were fixed in 1% glutaraldehyde and 1% OsO_4 at 4°C in 0.05 M cacodylate buffer (pH 7.4) for 4 h. Thick sections (1 μm) were stained with 1% methylene blue. Ultrathin sections (700 Å) of six random individuals from each group were examined with a Hitachi HU11E electron microscope operated at 75 kV. Light and electron micrographs are representative of various treatments or the control groups.

Statistical analysis

Statistical analyses were performed using a one-way analysis of variance with Student–Newman–Keals follow-up test.[35]

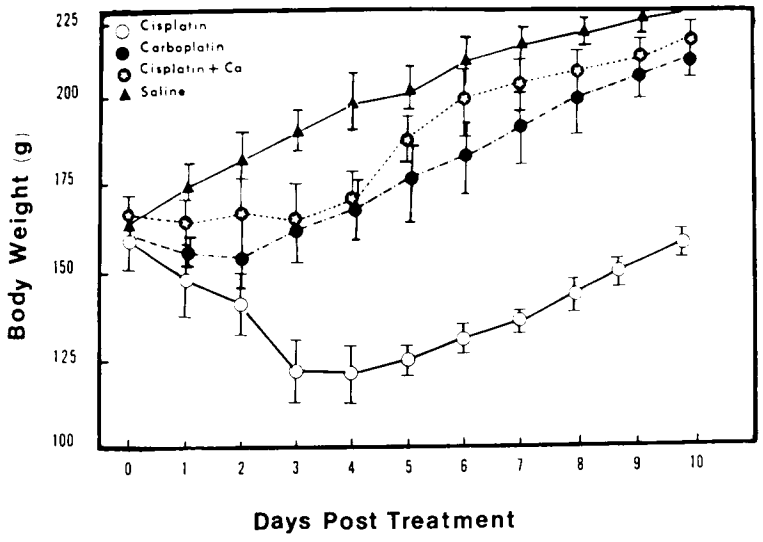

Figure 1. Body weight changes in Wistar rats following a single intraperitoneal injection of saline, cisplatin (7 mg/kg), carboplatin (50 mg/kg) or cisplatin (7 mg/kg) plus calcium (daily injection of 0.5 ml 10% calcium gluconate). Mean ± SD; $n = 10$ for each treatment group.

Results

Animal studies

Cisplatin-treated rats (Group I) had significant weight loss during the first week of treatment (Figure 1), with animals in higher dose groups (7–9 mg/kg) registering the greatest losses. Cisplatin plus calcium-injected animals (Group II) lost only a fraction of the weight as compared with the cisplatin-treated group. The animals in Group I continued to lose weight through the first 10 days of treatment while the cisplatin plus calcium-treated animals (Group II) began to gain weight by day 3. The control animals (Group III) gained weight throughout the duration of the experiment. The

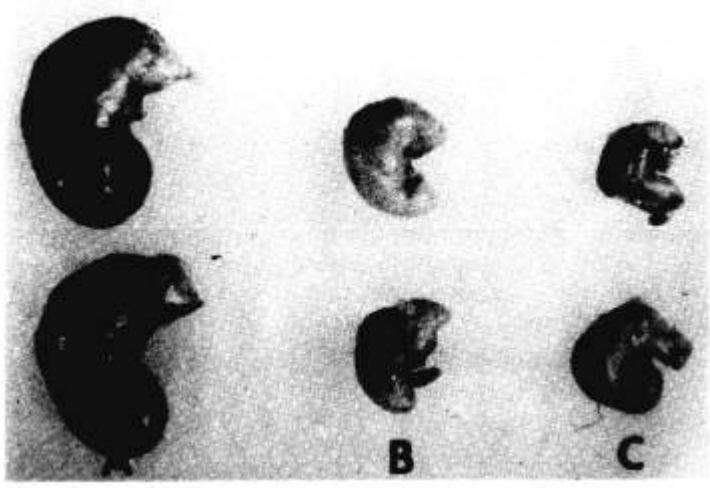

Figure 2. Stomachs from rats after 5 days of (A) cisplatin (Group I) treatment (7 mg/kg), (B) cisplatin (7 mg/kg) plus calcium (Group II) and (C) saline injected (0.85% NaCl). Note the distended stomachs resulting from cisplatin treatment (A). There is no distension when cisplatin-treated rats were given calcium.

carboplatin-treated (Group V) animals, after an initial no weight gain for about 2 days, continued to gain weight at an average rate. All of the cisplatin-treated animals (Group I) had noticeable gastric distension and ulceration (Figures 2 and 3) in the cardiac portion of the stomach by day 5. Gastric distension following cisplatin was primarily due to food retention. Table 2 shows that a single dose of 6 mg kg cisplatin induced the stomach distension, increasing the average weight of the stomach and its contents by more than 90% within 24 h to 300% by day 5. Higher dosages (9 mg/kg) were more effective in inducing gastric distension (average stomach mass = 550% of the controls 5 days later). Animals injected with calcium prior to or during cisplatin treatment (Group II) did not show any stomach bloating (Figure 2) or ulceration. The large intestinal contents of cisplatin-treated animals were very liquid, while those of cisplatin plus calcium-treated animals (Group II) had very formed fecal pellets. Large intestinal contents from normal animals given calcium (Group IV) were very formed and dry compared with the controls (Group III). A striking feature of cisplatin treatment in rats was a marked dose-related decrease in urine output by day 3, paralleled by a significant decrease in water intake (Figure 4). This urine

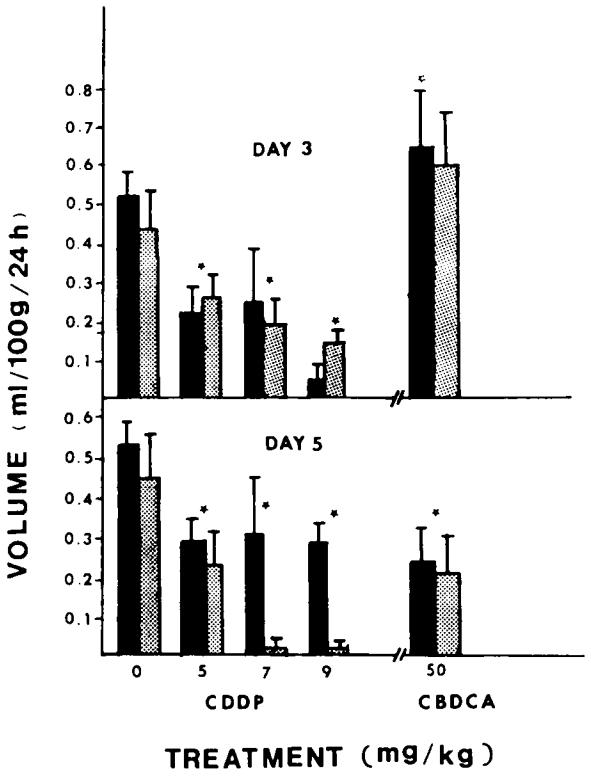

Figure 4. Relationship between the 24 h water intake (■) and urine output (▨) before and after cisplatin (5, 7 and 9 mg/kg) and carboplatin (50 mg/kg) treatments at day 3 and day 5. $n = 20$ per group; $^*p < 0.05$.

Figure 3. Dissected open stomach of a cisplatin-treated (7 mg/kg) rat (Group I) after 5 days of treatment showing the ulcerations (arrows) in the cardiac portion of the stomach.

output continued to decrease such that by day 5 there was little to no urine output, especially in higher dose groups (Group Ic). However, the water intake started to increase slowly after day 5 but it stayed lower than the normal animals through day 10. Carboplatin-treated animals (Group V) exhibited an increase in their water intake and urine output by day 3 but recorded a drop in both the parameters by day 5 (Figure 4). The water-deprived animals (Group VII) excreted negligible amounts of urine on any given day.

Morphological and cytochemical studies—pituitary

Morphological and cytochemical studies show that the axonal endings in the neurohypophysis contain neurosecretory granules that are best resolved as distinct granules in 1 μm thick sections stained with methylene blue (Figure 5A). Such granules are positive after PAS and chromalum hematoxylin. Under the electron microscope these granules appear highly electron dense and seem to arise by

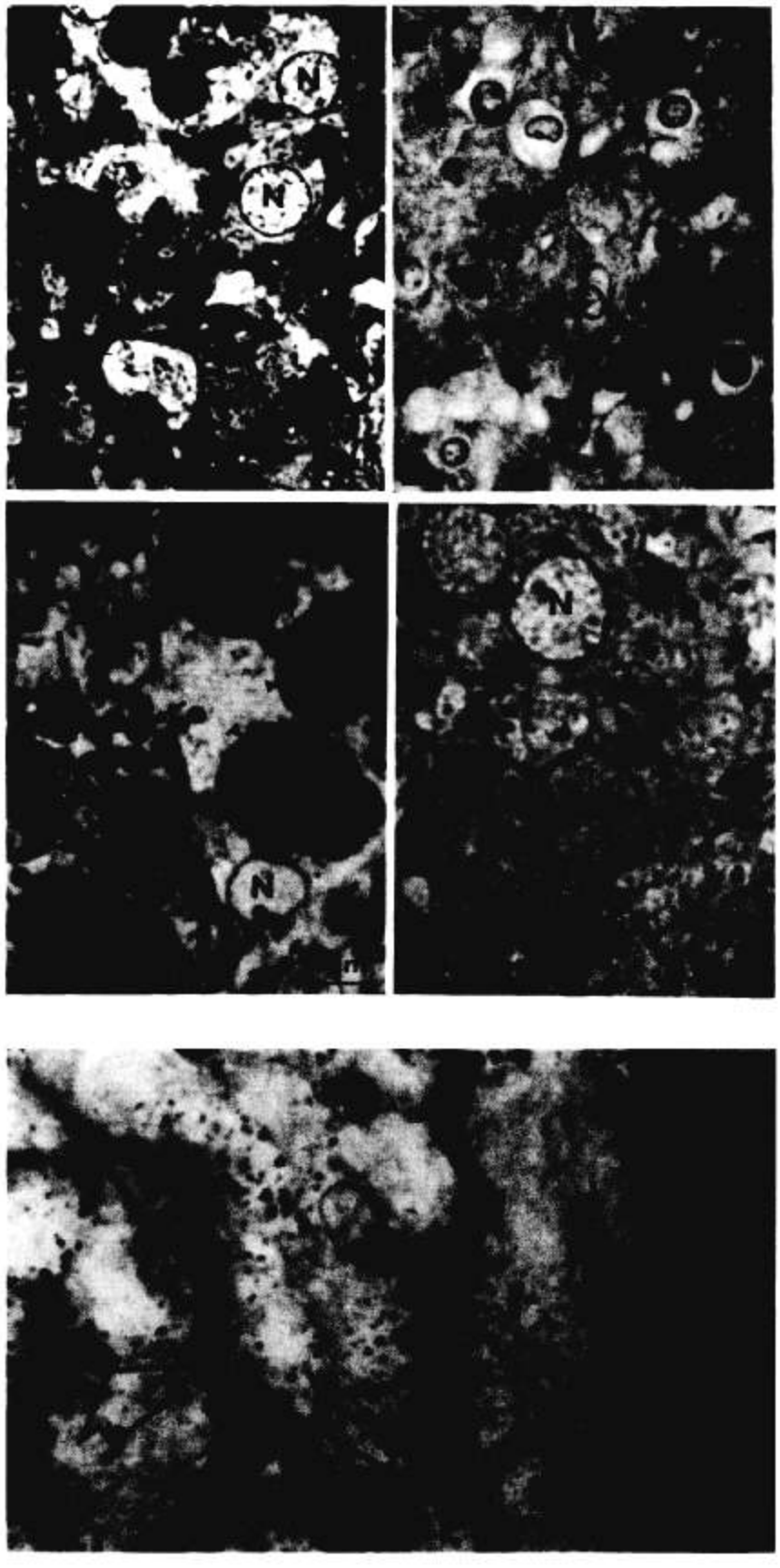

Figure 5. Light micrographs of neurohypophysis from normal (Group III), cisplatin-treated (Group I) and carboplatin-treated (Group V) rats showing the pituicytes and the axonal endings. (A) Normal neurohypophysis (Group III) after methylene blue staining showing the neurosecretory granules within the axonal endings (arrowheads). Note the pituicytes enclosing the axons (arrow). N, nuclei of the pituicytes. ×800. (B) Neurohypophysis from a 3 day cisplatin-treated (7 mg/kg) rat (Group I) after hematoxylin/eosin staining showing the absence of neurosecretory granules from the axonal endings. Note the rounded pituicytes (arrows). ×320. (C) Neurohypophysis from a 3 day carboplatin-treated (50 mg/kg) rat (Group V) after methylene blue staining showing an increase in the size and number of neurosecretory granules in the axonal endings (arrowheads). Note the large lipid globules (arrows) in the pituicytes. N, nucleus of a pituicyte. ×800. (D) Neurohypophysis from a 5 day carboplatin-treated (50 mg/kg) rat after methylene blue staining showing a complete absence of neurosecretory granules; however, the pituicytes are still not rounded as in the cisplatin-treated rats (see B). The lipid globules (arrowheads) still are present. n, nucleus. ×800.

Figure 6. Neurohypophysis from a 3 day cisplatin-treated (7 mg/kg) rat after PAS showing large deposits of glycogen (arrows) in the pituicytes. Note the extensive capillary network (arrowheads). I, intermediate lobe. ×320.

the fusion of smaller ones. Using immunocytochemical methods with a primary antibody against arginine vasopressin and a secondary antibody labeled with alkaline phosphatase it was possible to show a uniform staining of the neurosecretory granules in the axonal endings of the neurohypophysis in sections from a normal rat with no staining of the adjacent intermediate lobe or the anterior pituitary. After cisplatin treatment or after water deprivation, there was little to no staining of the neurosecretory granules indicating their discharge. Detailed results of these experiments have already been published and will not be repeated here.[34]

In addition to these changes in the axonal endings of the neurohypophysis cisplatin-treatment (Group I) induces some characteristic changes in the pituicytes. These include rounding of the pituicytes (Figure 5B) and large accumulations of PAS-positive material that tested positive for glycogen (Figures 6 and 7). Such accumulations of glycogen were very pronounced after 7 mg/kg of cisplatin treatment even after 3 days. At lower dose levels (5 mg/kg) glycogen accumulations were pronounced but only after 10 days of treatment. No such accumulations were observed after carboplatin treatment. Cisplatin treatment (Group I) or water deprivation (Group VII), in addition to causing loss of neurosecretory material from the axonal endings, induced a successive rounding up of the pituicytes which was most pronounced after 9 mg/kg treatment and after only 3 days of treatment. Such pituicytes lost their cytoplasmic extensions while most of their cytoplasmic organelles showed degeneration (Figures 5B and 8). This was characterized by swollen mitochondria, Golgi and the endoplasmic reticulum, and clumping of the nuclear material within the nucleus. Such cells were usually lost after about 10 days of treatment, leaving large vacuoles in the gland still observable 45 days post-treatment.

In the carboplatin-treated animals (Group V) after 3 days, the number and size of neurosecretory granules per axonal ending was greater as compared with controls. In a random section of a random gland from a normal rat there were 250 granules per unit area while in the carboplatin-treated animals there were 350 granules after 3 days of treatment. The size of such granules was also about 30% larger than the normal granules (Figure 5C). The pituicytes also showed a similar increase of about 30% in lipid droplets. After 5 days the number of lipid droplets further increased by about another 10%, but the amount of neurosecretory material in the axonal endings decreased to a point that no granules could be resolved with the light

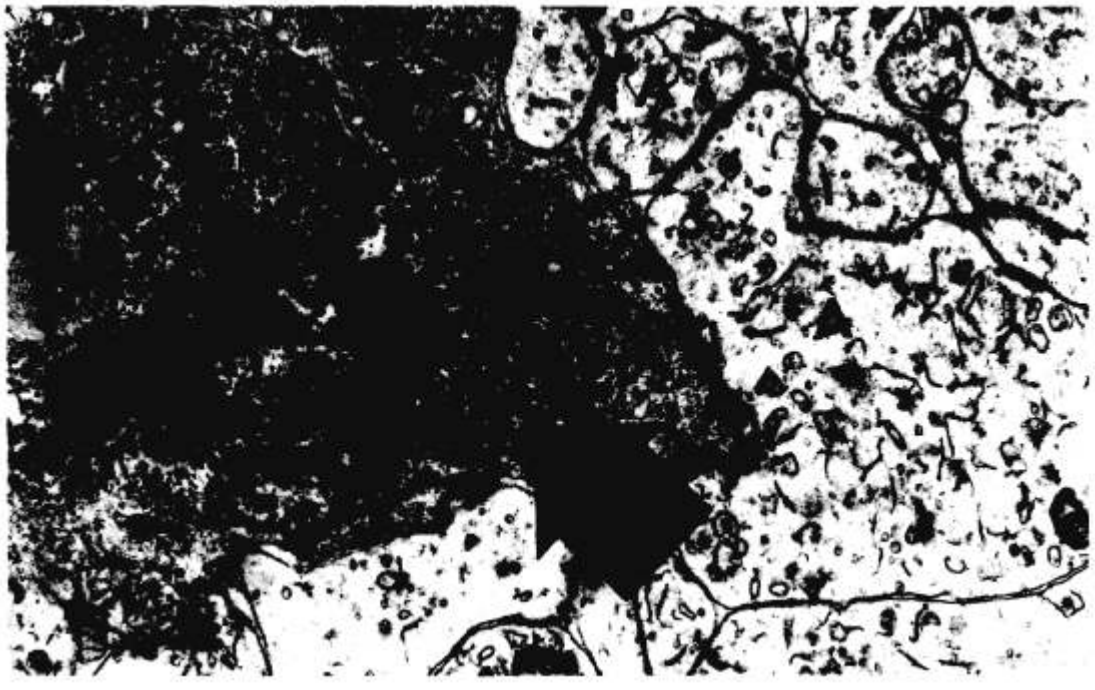

Figure 7. Electron micrograph showing glycogen (arrow) accumulations in the pituicytes and lack of neurosecretory granules from the axonal endings (A) after 3 days of cisplatin treatment. L, lipids. × 6000.

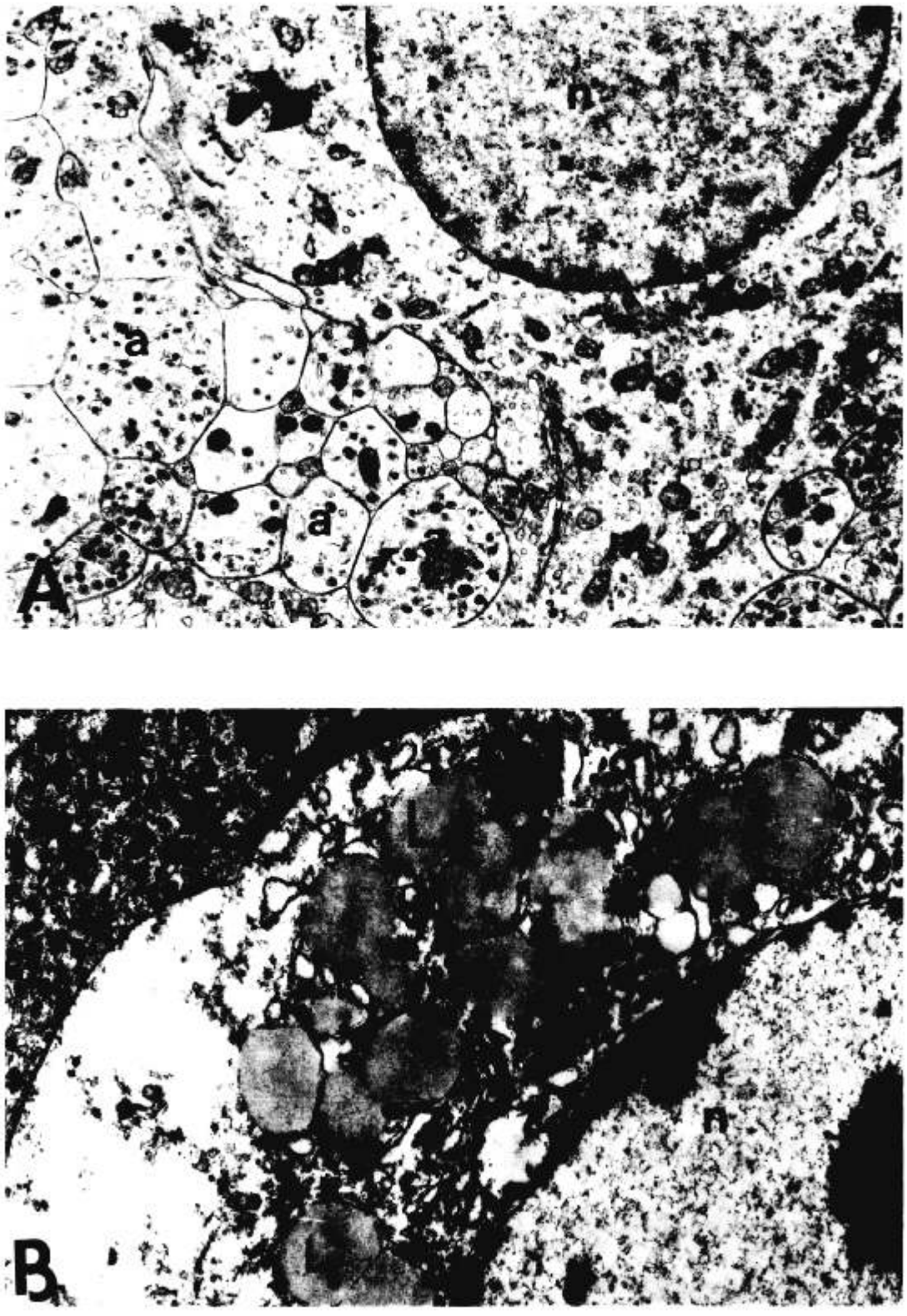

Figure 8. (A) Electron micrograph of a pituicyte from a normal (Group III) rat showing normal morphology and a uniform distribution of its organelles. (B) Electron micrograph of a pituicyte, as seen in Figure 5(B), after 3 days of cisplatin treatment (Group I). Note portion of the rounded up cell with disintegrated cellular components and accumulation of lipids (L). n, nucleus; nu, nucleolus; a, axonal endings; m, mitochondria; ×8000.

microscope (Figure 5D). This is very suggestive of a possible release of VSP from the posterior pituitary between 3 and 5 days of carboplatin treatment. This corresponds well with the decreased urine output for the same period (see Figure 4).

Alkaline phosphatase activity

Alkaline phosphatase activity in kidney sections from control animals (Group III) had most of the reaction product localized at the plasma membrane of the lumen border of the tubule cells (Figure 9). In a random section of the cortex, 20–25% of the viewing area was covered by the enzyme reaction product as measured by automatic image analysis.

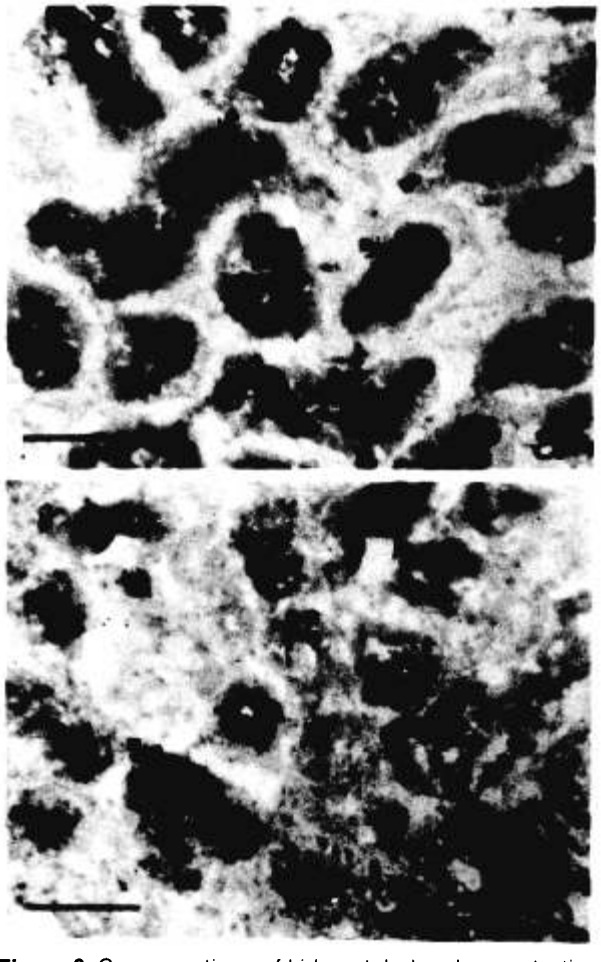

Figure 9. Cross-sections of kidney tubules demonstrating alkaline phosphatase activity (arrows) in (a) normal (Group III) and (b) 5 day cisplatin-treated (7 mg/kg) rats (Group I). Note in (b) the decreased reaction product at the lumen surface of the tubule cells after cisplatin treatment (arrows). Bar = 10 μm. ×650.

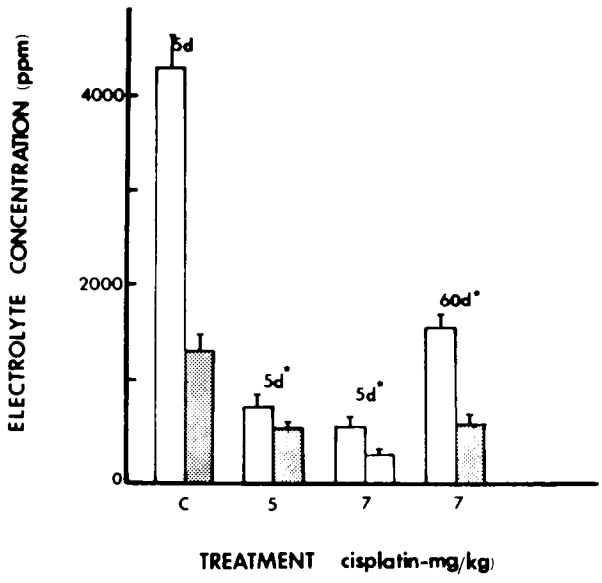

Figure 10. Calcium (□) and inorganic phosphate (▨) concentrations in the rat blood before (C, normal control) and after cisplatin treatment. Note the dramatic decline ($^*p < 0.05$) in the calcium and phosphate levels 5 days after treatment (5 and 7 mg/kg). This decline persisted up to 60 days post-treatment when the observations ceased. Standard error of the mean was less than 10% of the mean; $n = 10$ for each data point.

After 5 days of cisplatin treatment (Group I), however, 70–80% of the reactivity was lost, as compared with the control. Daily injections of calcium (Group II) seemed to protect the normal levels of alkaline phosphatase activity and prevented any weight loss or bloating of stomachs in the cisplatin-treated animals. Treating frozen sections of normal kidney with cisplatin (7–9 mg/100 ml saline solution) directly for upto 4 h had no effect on alkaline phosphatase activity both before or after a brief fixation in buffered formaldehyde (4% formaldehyde in cacodylate buffer, pH 7.2).

Blood plasma analysis demonstrated hypocalcemia and low phosphate levels in the cisplatin-treated (Group I) animals by day 5, and these low levels persisted through day 60 of treatment (Figure 10). However, cisplatin plus calcium-treated (Group II) animals showed normal levels of calcium (4200 p.p.m.) and phosphate (1200 p.p.m.) in their blood. Urine analysis showed fluctuations corresponding to the calcium injections. Urine analysis from cisplatin-treated (Group I) animals demonstrated a relatively low level of calcium after 5 days of treatment as compared with controls (Figure 11). Urine from normal but calcium-injected animals (Group IV) showed excessive accumulations of

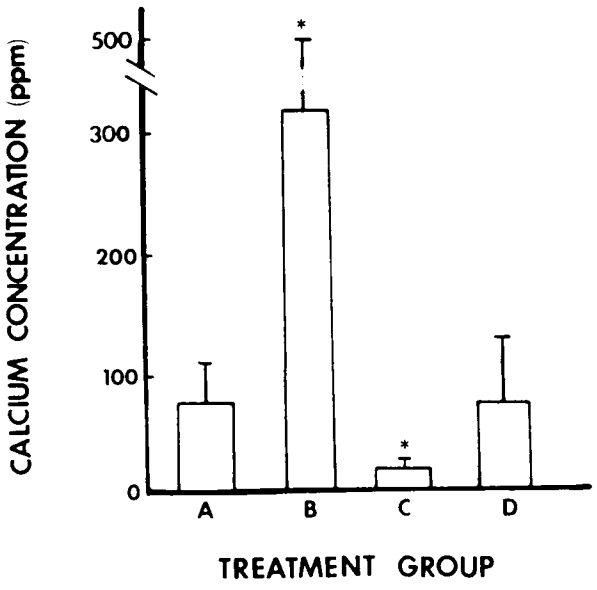

Figure 11. Urinary excretion of calcium in (A) control (Group III), (B) calcium-injected (0.5 ml of 10% calcium gluconate) (Group IV), (C) cisplatin-treated (7 mg/kg) (Group I) and (D) cisplatin-treated (7 mg/kg) plus calcium-injected (0.5 ml of 10% calcium gluconate) 5 days post-drug treatment (Group II). The difference in the excreted calcium between (A) the control and (B) the calcium-injected rat is equivalent to the amount of injected calcium. The decrease in C (Group I) corresponds to the very prominent hypocalcemia after cisplatin treatment ($n = 20$ for each treatment and control group). *$p < 0.05$.

calcium in their urine, almost equivalent to the amount injected. Cisplatin plus calcium-injected (Group II) rats on day 5 showed excretion of calcium in their urine comparable to the control animals (Group III).

Morphological studies—parathyroid gland

Parathyroid gland secretion (PTH) is responsible for maintaining calcium levels in the blood while its detailed morphology has already been reported[36,37] and will not be repeated here. In a normal gland there is a preponderance of relatively inactive light chief cells to actively secreting dark chief cells (Table 3). Computer image analysis of random micrographs of a normal gland shows the percentage of dark active secretory cells to be around 49.8 ± 5.8% (Figure 12). However, after cisplatin treatment (7 mg or 9 mg/kg) for 3 days the number of dark chief cells surpassed the light chief cells and by day 5 the percentage of dark cells was

Table 3. Effect of calcium upon cisplatin induced toxicities after 5 days of treatment

Treatment	Parathyroid gland morphology (percent active dark cells)
Saline alone	49.8 ± 5.8
Cisplatin (9 mg/kg)[a]	79.8 ± 10.6[b]
Cisplatin (9 mg/kg) + calcium (5 mg/day)	15.9 ± 12[c]

[a] Given intraperitoneally in 0.15 M NaCl on the starting day of the experiment.
[b,c] Significantly different from saline treatment ($p < 0.05$ and 0.01, respectively).

Figure 12. (A) A cross-section (1 μm) of a parathyroid gland from a normal rat stained with methylene blue showing the distribution of dark cells (arrows) amongst the light cells (L). (B) Parathyroid gland from a 3 day cisplatin-treated rat showing the preponderance of dark cells (arrows) to light cells (L). × 125.

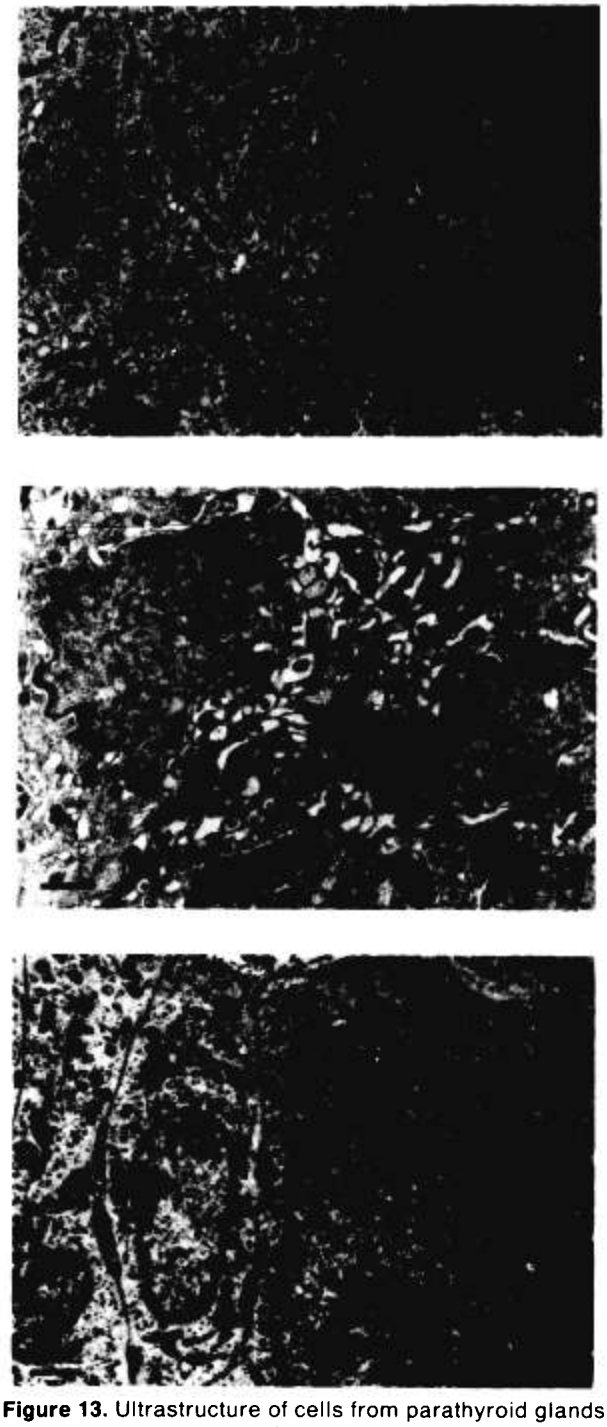

Figure 13. Ultrastructure of cells from parathyroid glands taken from (a) control (Group III), (b) cisplatin-treated (7 mg/kg) (Group I) and (c) cisplatin (7 mg/kg) plus calcium-injected (0.5 ml of 10% calcium gluconate) (Group II). (a) In the normal animals one can find light (L) and dark cells (D); however, in (b) after cisplatin treatment, dark cells predominate with increased nuclear membrane infoldings (arrows), extensive endoplasmic reticulum (ER) and Golgi (G). (c) Calcium injection of cisplatin-treated rats show mostly light chief cells with regular nuclear membrane, endoplasmic reticulum and Golgi. Bar = 1 μm.

observed to be 79.8 $\pm$ 10.6% ($p < 0.05$; also see Table 3). These dark cells are characterized by a highly involuted plasma membrane, extensive bloated rough-surfaced endoplasmic reticulum, well developed Golgi and a nucleus with a highly convoluted nuclear membrane (Figure 13). However, glands from cisplatin plus calcium-treated animals (Group II) were primarily composed of light chief cells with no involutions in their plasma or nuclear membranes (Figure 13). These cells demonstrated collapsed endoplasmic reticulum, Golgi and few mitochondria. There were almost one-seventh of the dark cells (15.9 $\pm$ 12; $p < 0.01$) in such glands as compared with the controls (Group III).

Discussion

Although cisplatin has been on the market for the treatment of ovarian and testicular tumors for over a decade, its mechanism of action is far from clear. In order to improve its therapeutic index and lower its associated nephrotoxicity several cisplatin analogs have been synthesized and tested.[38] So far no compound has been able to match the therapeutic efficacy of cisplatin inspite of cisplatin-associated severe nephrotoxicity—a major dose-limiting factor in its use. Various clinical strategies have been employed to combat its nephrotoxicity; however, diuretic therapy has proven to be the method of choice.[30] The kidney's vulnerability to cisplatin may stem from its ability to accumulate and retain cisplatin to a greater degree than other organs[29] and diuresis probably decreases urinary platinum concentration. Cisplatin has been shown to be diuretic in Sprague-Dawley[34,40] and Long-Evans[28] rats, and initially less nephrotoxic as compared with Wistar rats, where it is antidiuretic and nephrotoxic.[28] Immunocytochemical and morphological evidence demonstrates that the change in the urine output of the Wistar rats after cisplatin treatment correlates well with the release of VSP from the posterior pituitary.[34,41,42] In contrast, carboplatin treatment of Wistar rats showed an increase in the neurosecretory granules in the posterior pituitary for the first 3 days. There is a corresponding increase in the urine output during these 3 days which probably would result in a greater excretion of carboplatin. Indeed, 90% of the injected carboplatin has been documented as excreted in the urine as compared with only 50% of cisplatin for the same time.[29] Up to this time there is little associated nephrotoxicity due to

carboplatin treatment.[28] However, around day 5 of carboplatin treatment, a release of neurosecretory granules from the pituitary with a corresponding decrease in urine output is observed. This is probably responsible for an increased accumulation of platinum within the kidney cells, resulting in high lysosomal activity and lysis.[28,43]

Like mercury,[44] cisplatin has also been shown to affect mitochondrial respiration.[14] In studies involving isolated mitochondria, cisplatin has been shown to cause a release of stored Ca^{2+} leading to the uncoupling of oxidative phosphorylation.[14,45,46] Such a release of Ca^{2+} from the intracellular stores would result in increased cytosolic Ca^{2+} levels. Increased cytosolic calcium has been suggested to cause the depolymerization of intermediate microfilaments leading to the inhibition of cytokinesis, which could be one of the mechanisms to block tumor growth.[23,24] Increased cytosolic calcium has also been shown to cause an increase in gluconeogenesis in the isolated kidney tubules.[47] Indeed, accumulations of glycogen have been demonstrated in proximal tubule cells after cisplatin treatment,[45] and in a number of other diverse tissues, such as the neurohypophysis[48] and lymphocytes,[49] supporting the hypothesis that the increased cytosolic calcium is responsible for these glycogen accumulations after cisplatin. Cisplatin treatment caused hypocalcemia in animals and in clinical studies.[27] Low serum calcium levels have been associated with a number of conditions including bloating of stomachs in the rat.[14] Stomach smooth muscle from such animals has been shown to be hypercontractile to acetylcholine stimuli in a dose-related fashion.[45,50] Such animals have also been noted to show delayed rigor mortis.[14] Injecting calcium into cisplatin-treated rats prevents bloating of the stomach and restores normal contractility of the smooth muscle. There seems to be a block in the release of acetylcholine from the nerve endings which is calcium dependent and may be a possible reason for the neurotoxicity associated with the drug.[51]

Calcium is important for the activity of several enzymatic systems, including those responsible for muscular contraction like Ca^{2+}-ATPase, and transmission of nerve impulses. Changes in the motility of the gastro-intestinal tract, uterine smooth muscle or blood vessels require altered control of calcium ions.[52] Various signals responsible for the contractions of the smooth muscle interact with the receptors on the cellular membrane to activate the channels for entry or release of sequestered calcium.[53] Transport enzymes are of major biological importance in the kidney and elsewhere throughout the organism.[54] Changes in transport enzymes would also result in intracellular ion concentrations with altered cell functions. Such changes in the protein molecules responsible for intracellular calcium homeostasis may be one of the reasons for a reduced drug efflux from the cells modulated by the calcium antagonists.[55] Various calcium channel blockers (verapamil, nifedipine) and calmodulin antagonists (chlorpromazine) have been used to enhance the antitumor action of various chemotherapeutic agents including cisplatin[56-60] through their interaction with various membrane glycoproteins to block Ca^{2+} efflux. The use of calcium channel blockers like verapamil has been demonstrated to be helpful to reverse multidrug resistance.[61,62]

Normally, the concentration of calcium in the blood is quite stable because of the mobilization of calcium due to the action of hormones on bones. Parathyroid gland hormone activates the osteoclasts, promoting resportion of the bone matrix with liberation of calcium and phosphate ions into the blood. Indeed, cisplatin induces morphological changes in the parathyroid gland characteristic of drugs that cause low serum calcium.[37,48] Injecting calcium before the start of cisplatin treatment has a protective effect on parathyroid gland morphology, and amelioration of various toxic side-effects like bloating of the stomach and loss of transport enzymes like alkaline phosphatase from the cell membranes.[43]

Conclusion

From the present investigations it seems that the various toxicities associated with cisplatin may not be the result of its direct action on various organ systems but are the indirect result of calcium changes in the body. Calcium supplements before and during cisplatin treatment seem to protect the kidney. It remains to be seen how the Ca^{2+} and the VSP receptors on kidney cells are effected by cisplatin or carboplatin treatment so as to cause diuresis or hypocalcemia. However, it is certain that different analogs of cisplatin have proven to influence kidney function differently in the same strain. Thus, in choosing animals for toxicological studies, strain specific differences regarding sensitivity to specific drugs must be considered in the experimental design and the presentation of data.

Acknowledgments

Part of this work was done under a Fulbright Exchange program at M. S. University of Baroda. Thanks are due to Drs RL Pax and Clifford W Welsch for the critical evaluation of the manuscript, and to Mrs Christine Keyes for secretarial help. The drugs cisplatin and carboplatin were a gift of Bristol-Meyer Company and the National Cancer Institute.

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(Received 5 January 1993; accepted 3 February 1993)