Exposure to excessive parenteral Zn and/or cisplatin affects trace element metabolism and enzyme activities in reproductive tissues and kidney of male rats

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Cisplatin (CDDP) is a platinum coordination compound with antineoplastic properties. Cisplatin chemotherapy is known to cause serious damage to the kidney tubules and to the germinal epithelium of the testis. Results of recent investigations suggest that pretreatment with parenteral Zn will prevent some of the damage in these tissues. The present study determined the effects of pretreatment with large doses of parenteral Zn on trace element metabolism and enzyme activity in CDDP-treated rats. Treating young male rats (200 g) with intraperitoneal injections of 90 mg Zn/kg body weight prior to CDDP treatment (3 mg/kg body weight for 3 days) did not inhibit kidney damage; confirmed by elevated urea and creatinine concentrations in serum of CDDP-treated rats. Serum and testicular testosterone concentrations were significantly lower in CDDP-treated rats than controls, which suggests Leydig cell damage. Zn treatment did not, however, alleviate this effect. Although angiotensin-converting enzyme activity was significantly elevated in the kidney by Zn treatment, activities in the testis and epididymis were not affected. Contrary to the suggestions that high parenteral Zn treatment protects kidneys and testes from damage, this study shows no such positive effects. However, the study demonstrated that treating rats with high concentrations of Zn and/or CDDP caused numerous changes in tissue concentrations of zinc, copper, and iron and in the activities of Zn-dependent as well as non–Zn-dependent membrane-bound enzymes.

Keywords: zinc; copper; iron; metallothionein; angiotensin converting enzyme; testis; epididymis; kidney

Introduction

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is a platinum coordination compound with antineoplastic properties and is used extensively in the treatment of solid tumors of various types.[1] CDDP chemotherapy, however, is known to cause serious damage to the kidney tubules[2] and to the testis germinal epithelium.[3] In the testis, it leads to conditions that suggest that the Leydig and Sertoli cells are dysfunctional; testosterone and androgen binding protein productions, respectively, are reduced.[4]

Experiments have shown that prior treatment of mice with trace elements such as Cu, Se, and/or Zn protects the kidneys from damage caused by subsequent treatment with CDDP.[5–7] This suggests that animals or humans with low trace element status might be more susceptible to the toxic side-effects of this drug than those with adequate status. Recent studies have shown, however, that the protective effects of Zn and Cu found in mice apparently are not present in all species. Reeves et al.[8–10] showed that the kidneys

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Received October 3, 1991; accepted January 30, 1992.

of rats pretreated with large doses of dietary Zn or Cu and parenteral Zn were not protected from CDDP.

Pogach et al.[4] showed, however, that pretreatment of adult rats with high doses of parenteral Zn partially ameliorated the effects of CDDP on Sertoli cells. This was shown by the maintenance of near normal concentrations of androgen-binding protein in the testes of drug-treated rats that had received prior treatment with Zn. Subsequent studies showed that germ cell deterioration was still present 10 days following a 5-day treatment with CDDP.[11]

Angiotensin converting enzyme (EC 3.4.15.1, ACE), a Zn-dependent enzyme, is present in high concentrations in mature germinal cells.[12] Its change in activity under chemical or physiological stress might be used as a marker for the cell's viability. Recently, Reeves[13] showed that rats deprived of Zn in the early stages of sexual development develop hypogonadism and have a reduced activity of testicular and epididymal ACE.

Reeves et al.[14] also showed that CDDP treatment elevated ACE activity in kidney microvilli of rats, and the increase was associated with an increase in tissue concentration of Zn. Other Zn-dependent enzymes including alkaline phosphatase and endopeptidase, 24-11 were depressed by the drug. The activity of another membrane-bound, non–Zn-dependent enzyme, gamma-glutamyl transferase, was depressed. Kidney concentrations of Cu and Fe were also affected by CDDP. It was of interest, therefore, to determine if CDDP treatment affects trace element metabolism and enzyme activity in the male reproductive organs of Zn-treated rats as it does in the kidney.

Materials and methods

Animals

Sixty young male Sprague-Dawley rats (170 ± 10 grams) were purchased from Sasco Laboratories, Lincoln, NE, USA. They were housed in an air-conditioned animal room with a 12-hr light/dark cycle and fed a semi-purified diet similar to that used before[13] except that the standard AIN-76 mineral mix (Teklad, Madison, WI, USA; TD170915) was used instead of the modification (Teklad, TD87282). The diet contained an adequate supply of all nutrients known to be essential for the rat.

Experimental protocol

After 7 days, rats were randomly divided into two groups of 30 rats each. Each rat in one group was given a subcutaneous injection of an emulsion of sunflower oil and Zn carbonate. The dose was equivalent to 90 mg Zn/kg body weight (BW). It was given every other day as 1 mL of emulsion per kg BW for the duration of the experiment. Rats in the other groups received injections of oil only. This procedure was used to simulate continuous Zn absorption by allowing the slow release of Zn into the circulation.

After 7 days on the above regimens, each group (the Zn-injected group and those not injected) was randomly divided into three subgroups of 10 rats each. These consisted of a control group, a CDDP-treated group, and a group pair-fed to the CDDP-treated rats. It has been a consistent observation that CDDP treatment causes rats to reduce their food intake. To account for the possible effects of reduced food intake, individual rats in the pair-fed group were given the same amount of diet eaten the previous day by their paired mates in the CDDP-treated group. CDDP treatment consisted of giving each rat an intraperitoneal injection of 3 mg CDDP in saline/kg BW each day for 3 consecutive days. All other rats received injections of saline.

Termination of experiment

Sixty-two hours after the start of CDDP treatment, rats from the control and CDDP-treated groups were fasted from 11:00 p.m. until 9:00 a.m. the next morning. Then they were anesthetized with intraperitoneal injections of sodium pentobarbital, 50 mg/kg BW. Rats in the pair-fed group were fasted and anesthetized the following day. Blood was drawn from the abdominal aorta into Monovette tubes (Sarstedt, Newton, NC, USA) and allowed to clot at room temperature for 1 hour. The blood was centrifuged at 1500*g* for 20 min and the resulting serum frozen until analyzed. The kidneys, testes, and one epididymis were excised and frozen.

The second epididymis was excised and minced into pieces in a small petri dish. The sperm were removed from the pieces with three consecutive 0.5 mL washes of 145 mmol/L NaCl. Between washes, the petri dish was rocked to dislodge the sperm and the solution containing the sperm was removed with a transfer pipet. Each sample was diluted appropriately and the sperm counted manually with the use of a hemocytometer and a phase contrast microscope. The remaining epididymal pieces were stored frozen at −20° C until further analysis.

Trace element analysis

Proteins were precipitated from 0.5 mL of serum with 0.5 mL of sulfosalicylic acid in water (46 mmol/L) and an additional 1.0 mL of deionized water. The mixture was allowed to sit at room temperature for 1 hour and then centrifuged at 2000*g* for 20 min. The resulting supernatant was analyzed for Zn, Cu, and Fe by flame atomic absorption spectroscopy (AAS); Pt was determined by furnace AAS. Portions of a kidney and a testis and a whole epididymis were lyophilized and the dry tissue weighed to the nearest 0.1 mg. Samples were dry-ashed at 450° C for 48 hrs in a muffle furnace; the resulting ash was dissolved in 5 mL of 100 mmol/L HCl. These samples were analyzed for Zn, Fe, and Cu by flame AAS and for Pt by furnace AAS.

Testosterone analysis

Serum and testis testosterone concentrations were determined by radioimmunoassay (RIA). The RSL Testosterone-^{3}H Kit from ICN Biomedicals, Inc. (Costa Mesa, CA, USA) was used. A portion of a testis was homogenized (tissue:buffer, 1:5, wt/vol) in 400 mmol/L NaCl, 50 mmol/L HEPES buffer, pH 7.0, with a Tekmar homogenizer. Testosterone was extracted from an aliquot of each homogenate and from freshly thawed serum with a mixture of n-hexane and ethyl acetate (2:3, vol/vol). The extraction procedure was carried out at room temperature for 30 min. After centrifugation, the organic layer was removed and dried under a stream of nitrogen gas. The residue was resuspended in assay buffer and diluted appropriately for the RIA procedure. Recovery of testosterone was monitored by the addition of a known amount of ^{3}H-testosterone to each tube; recovery was consistently greater than 95%.

Enzyme analysis

Alkaline phosphatase (EC 3.1.3.1, AP) and gamma-glutamyl transferase (EC 2.3.2.2, GGT) activities were determined in testicular, epididymal, and kidney tissues. Tissues were homogenized in the NaCl-HEPES buffer described above and analyzed for AP and GGT activities with kits 545 and 104, respectively, from Sigma Chemicals, St. Louis, MO, USA.

Membrane-bound ACE activity was also determined in the testis, epididymis, and kidney. A portion of each tissue was homogenized in the NaCl-HEPES buffer and centrifuged at 40,000*g* for 40 min. The supernatant was discarded and the pellet resuspended in a volume of buffer equal to that of the previous supernatant. After centrifuging the sample again, the pellet was resuspended in a similar amount of NaCl-HEPES buffer containing 10 μmol/L $ZnCl_2$ and Triton-X 100 (7.7 mmol/L). The membrane proteins were solubilized by incubating the mixture on ice and vortexing it every 15 min for 3 hours. The samples were centrifuged at 40,000*g* for 40 min and the supernatant analyzed for protein content with the bicinchoninic acid method.[15] ACE activity was determined by the method of Neels et al.[16] as modified by Reeves and O'Dell.[17]

Other analyses

Fresh serum was analyzed for creatinine and urea nitrogen concentrations by procedures outlined in Sigma Kits 555 and 535, respectively. Kidney and testis samples were analyzed for metallothionein (MT) content by the ^{109}Cd displacement method of Eaton and Toal.[18] We used the same procedures they did except that 10 mmol/L 2-mercaptoethanol was added to the homogenizing buffers.

The data were analyzed by the analysis of variance (ANOVA) procedures of Crunch Software Corporation, Oakland, CA, USA (Version 4, Beta Release). Hartley's F_{MAX} test[19] for homogeneity of variances was done with each ANOVA. When the test was significant, that is, variances were not homogeneous, the data were transformed to achieve or to approach homogeneity. Data for each transformed variable are presented in appropriate tables. Significant differences between group means were determined by a step-down multiple stage *F* test (REGWF).[20–22]

Results

Table 1 shows the effects of CDDP and Zn treatment on food intake and body weight, and testicular, epididymal, and kidney weights. All rats began the CDDP treatment at similar weights. However, because CDDP depressed food intake, these rats lost about 11% of their original body weight during the 3 days of treatment. All rats in the CDDP-treated group had food in their stomachs at the time they were killed. This occurred even though they ate less than 1 g of diet before beginning the 9-hr fasting period (data not shown), which suggests that stomach emptying and gut transit time were greatly reduced. Control or pair-fed rats had no food left in their stomachs after the 9-hr fast. Although the pair-fed rats ate the same amount of food as the CDDP rats during the 3-day treatment period, their loss in body weight was only 6%. Zn injections had no effect on body weight. There was not a significant effect of CDDP treatment or Zn injections on weight change in the testis.

The effects on the epididymis and kidney weights were complex *(Table 1)*. Cisplatin treatment lowered epididymis weight but only relative to the pair-fed group not treated with Zn. Treatment with the drug increased kidney weight compared with the controls and pair-feds, but only in the group not treated with Zn.

Table 2 shows the effects of CDDP and Zn injections on the serum parameters that were measured. The indicators of kidney malfunction, serum urea and creatinine concentrations, were significantly ($P < 0.001$) elevated in CDDP-treated rats. Zn injections had no effect on ameliorating these responses, however. Zn injections elevated serum Zn three-fold and there was a tendency for CDDP alone to elevate serum Zn. CDDP treatment caused a significant elevation in serum Fe in both Zn-treated groups *(Table 2)*. Zn treatment, on the other hand, depressed serum Fe. Serum Cu

Table 1 Effect of CDDP treatment and pair feeding on body weight, food intake, and tissue weights in Zn-injected rats[1]

	Not Zn-injected			Zinc-injected			ANOVA table		
	Control	CDDP	Pair-fed	Control	CDDP	Pair-fed	Zn	Treat	Zn × T
Body, g[2]	246 ± 4	218 ± 5	238 ± 5	247 ± 5	220 ± 5	233 ± 5	NS[3]	0.001	NS
Food intake, g/d	13 ± 0.3	5 ± 0.3	5 ± 0.3	14 ± 0.5	6 ± 0.5	6 ± 0.5	NS	0.001	NS
Testis, g	1.35 ± 0.04	1.36 ± 0.06	1.42 ± 0.04	1.35 ± 0.06	1.35 ± 0.06	1.42 ± 0.05	NS	NS	NS
Epididymis, g	0.28 ± 0.03[a]	0.28 ± 0.01[a]	0.32 ± 0.01[b]	0.29 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	NS	0.003	0.030[4]
Kidney, g	0.82 ± 0.03[a]	0.95 ± 0.03[b]	0.85 ± 0.04[a]	0.92 ± 0.04[b]	0.89 ± 0.03[b]	0.80 ± 0.03[a]	NS	0.012	0.027

Pairwise comparisons of means for factor treatment[5]

	Body wt.	Food intake	Epididymis wt.	Kidney wt.
Control	a	a	a	ab
CDDP	b	b	a	a
Pair-fed	c	b	b	b

[1]Values represent means ± SEM of 10 replicates.
[2]Body weight represents the final body weight. Organ weights represent one organ taken at the termination of the experiment.
[3]NS, not significant.
[4]When an interaction occurred, significant differences between comparable groups were determined. Treatments with different letters within variables are significantly different from each other with a *P* value of 0.05 or less.
[5]Treatments with different letters within variables are significantly different from each other with a *P* value of 0.05 or less.

Table 2 Effect of CDDP treatment and pair feeding on serum parameters in Zn-injected rats[1]

Original data	Not Zn-injected			Zinc-injected			ANOVA table		
	Control	CDDP	Pair-fed	Control	CDDP	Pair-fed	Zn	Treat	Zn × T
Urea, mmol/L	2.2 ± 0.4	36.7 ± 6.3	2.0 ± 0.2	2.1 ± 0.2	40.5 ± 3.1	3.3 ± 0.5			
CRT, μmol/L	75 ± 2	267 ± 33	81 ± 2	72 ± 3	293 ± 18	76 ± 2			
Zn, μmol/L	17.3 ± 0.3	18.4 ± 0.8	16.8 ± 0.3	52.7 ± 4.0	53.8 ± 4.1	44.6 ± 2.0			
Cu, μmol/L	17.1 ± 0.5	16.8 ± 0.5	15.4 ± 0.9	22.6 ± 0.9	23.8 ± 1.0	21.9 ± 1.1	0.001	NS	NS
Fe, μmol/L	30.4 ± 1.7	41.3 ± 3.5	33.1 ± 2.0	22.5 ± 1.3	33.0 ± 2.0	20.5 ± 1.3	0.001	0.001	NS
TT, nmol/L	12.5 ± 2.9	1.3 ± 0.3	6.9 ± 3.9	6.7 ± 1.0	1.5 ± 0.2	4.0 ± 0.9			
Ln-transformed data									
Urea	0.68 ± 0.17	3.42 ± 0.23	0.66 ± 0.11	0.72 ± 0.08	3.67 ± 0.08	1.01 ± 0.22	NS	0.001	NS
CRT	4.31 ± 0.02	5.50 ± 0.15	4.39 ± 0.03	4.27 ± 0.03	5.67 ± 0.08	4.33 ± 0.03	NS	0.001	NS
Zn	2.85 ± 0.02	2.91 ± 0.04	2.82 ± 0.02	3.94 ± 0.08	3.96 ± 0.08	3.79 ± 0.04	0.001	0.062[2]	NS
TT	2.30 ± 0.24	0.05 ± 0.24	1.89 ± 0.14	1.82 ± 0.19	0.34 ± 0.13	1.22 ± 0.23	NS	0.001	0.048

Pairwise comparisons of means for factor treatment[3]

	Urea	CRT	Zn	Fe	TT
Control	a	a	a	a	a
CDDP	b	b	b	b	b
Pair-fed	a	a	a	a	c

[1]Values represent means ± SEM of 7–10 replicates. CRT, creatinine; TT, testosterone; NS, not significant.
[2]To show that the probability of significance difference was close to 95%.
[3]Treatments with different letters within variables are significantly different from each other with a *P* value of 0.05 or less.

Table 3 Effect of CDDP treatment and pair feeding on testis parameters in Zn-injected rats[1]

Original data	Not Zn-injected			Zinc-injected			ANOVA table		
	Control	CDDP	Pair-fed	Control	CDDP	Pair-fed	Zn	Treat	Zn × T
Zn, μmol/kg[2]	2658 ± 55	2650 ± 50	2362 ± 78	2626 ± 47	2562 ± 114	2654 ± 63	NS	NS	NS
Cu, μmol/kg	187 ± 4	184 ± 4	186 ± 4	188 ± 5	184 ± 3	190 ± 4	NS	NS	NS
Fe, μmol/kg	1539 ± 42	1525 ± 64	1567 ± 68	1388 ± 85	1407 ± 30	1493 ± 37	0.018	NS	NS
Pt, μmol/kg	—	52 ± 6	—	—	51 ± 7	—	NS	—	—
MT, μmol/kg	9.3 ± 0.3	9.2 ± 0.4	8.9 ± 0.4	9.6 ± 0.5	9.6 ± 0.4	8.9 ± 0.2	NS	NS	NS
TT, nmol/kg	398 ± 61	111 ± 15	342 ± 53	370 ± 68	76 ± 9	250 ± 63			
ACE	46.3 ± 2.7	42.9 ± 2.7	48.3 ± 1.4	44.0 ± 2.9	46.7 ± 3.3	46.7 ± 1.5	NS	NS	NS
AP	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	NS	NS	NS
GGT	9.5 ± 0.4	10.9 ± 1.2	9.0 ± 0.6	10.6 ± 0.9	10.3 ± 0.4	10.4 ± 0.7	NS	NS	NS
Ln-transformed data									
TT	5.9 ± 0.2	4.6 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	4.3 ± 0.1	5.2 ± 0.3	0.045	0.001[3]	NS

[1]Values represent means ± SEM of 7–10 replicates. MT, metallothionein; TT, testosterone; ACE, angiotensin converting enzyme, μkat/mg protein; AP, alkaline phosphatase, nkat/mg protein; GGT, gamma-glutamyl transferase, pkat/mg protein; NS, not significant.
[2]On a dry-weight basis.
[3]Pairwise comparisons of means for factor treatment: control, a; CDDP, b; pair-fed, a. Groups with different letters are significantly different from each other with a *P* value of 0.01 or less.

concentrations were elevated by Zn treatment but they remained unaffected by CDDP.

With regard to serum testosterone concentrations, there was a significant interaction between Zn and the type of treatment. The concentration of testosterone in serum of CDDP-treated rats was reduced to only 15% of the combined mean of the control and pair-fed rats *(Table 2)*. Serum testosterone was also slightly depressed by Zn treatment but, because of large variations between subjects, there were no significant differences.

Very few of the testis parameters measured were affected by CDDP or Zn treatment *(Table 3)*. Testosterone concentration was an exception. Cisplatin treatment reduced it to approximately 28% of the control or the pair-fed values. The drug did not affect any of the other parameters measured. Testosterone was also significantly lower in the testis of Zn-treated rats than in those not treated with Zn. The Fe concentration in testis was significantly lower in the Zn-treated rats than those not treated. Zn treatment alone had no effect on testis Zn or Cu concentrations. The activities of none of the enzymes measured were affected by either treatment. Metallothionein concentrations in the testis also were unaffected by CDDP or Zn treatment.

As in the testis, most of the parameters measured

in the epididymis were unaffected by CDDP *(Table 4)*. However, unlike the testis, the epididymis was affected by Zn treatment. The Zn concentration of the epididymis was lower ($P < 0.06$) in those rats injected with high doses of Zn than in those not injected. Cu concentrations were also lower in the Zn-treated rats. Overall, the number of sperm in the epididymal ducts was depressed by 21% in Zn-treated rats compared with those not treated. Epididymal AP activity was also depressed by Zn treatment but ACE and GGT activities were unaffected.

Most of the kidney parameters measured were significantly affected by both Zn and CDDP treatments *(Table 5)*. Regardless of Zn treatment, CDDP treatment elevated kidney Zn and Fe while lowering the Cu concentration. However, pair feeding also tended to elevate Zn and Fe in the kidneys of rats treated with Zn. Metallothionein concentrations tended to follow those of Zn, but because of large variation, there were no significant differences between controls, CDDP, and pair-fed groups. However, there was a two-fold higher MT concentration in kidneys of rats treated with Zn compared with those not treated.

While ACE activity was not affected by Zn treatment in both the testis and epididymis, it was elevated by 56% in the kidney. ACE activity was also higher in the kidneys of rats treated with CDDP than in those not treated. Alkaline phosphatase activity was not significantly affected by Zn treatment but was depressed by about 47% in CDDP-treated rats. Gamma-glutamyl transferase activity in the kidney was likewise lower by about 46% in CDDP-treated rats compared with those not treated.

Table 4 Effect of CDDP treatment and pair feeding on epididymis parameters in Zn-injected rats[1]

	Not Zn-injected			Zinc-injected			ANOVA table		
	Control	CDDP	Pair-fed	Control	CDDP	Pair-fed	Zn	Treat	Zn × T
Zn, μmol/kg[2]	2449 ± 127	2389 ± 128	2416 ± 112	2244 ± 134	2250 ± 117	2185 ± 104	0.062[3]	NS	NS
Cu, μmol/kg	122 ± 6	119 ± 4	119 ± 3	97 ± 9	114 ± 7	116 ± 4	0.030	NS	NS
Fe, μmol/kg	1068 ± 60	1010 ± 107	1128 ± 58	1079 ± 65	1023 ± 60	1100 ± 90	NS	NS	NS
Pt, μmol/kg	—	18 ± 2	—	—	19.5 ± 2	—	NS	—	—
Sperm CT	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	0.020	NS	NS
ACE	20.1 ± 3.4	21.6 ± 3.1	25.0 ± 2.7	23.0 ± 2.2	22.6 ± 2.0	21.1 ± 1.9	NS	NS	NS
AP	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	0.002	NS	NS
GGT	5.9 ± 0.3	6.0 ± 0.3	6.5 ± 0.3	6.1 ± 0.4	5.7 ± 0.1	6.4 ± 0.2	NS	NS	NS

[1]Values represent means ± SEM of 7–10 replicates. Sperm CT, sperm count × 10^8/g epididymis; ACE, angiotensin converting enzyme, nkat/mg protein; AP, alkaline phosphatase, nkat/mg protein; GGT, gamma-glutamyl transferase, nkat/mg protein; NS, not significant.
[2]On a dry-weight basis.
[3]To indicate that the probability of significance was close to 95%.

Table 5 Effect of CDDP treatment and pair feeding on kidney parameters in Zn-injected rats[1]

Original data	Not Zn-injected			Zinc-injected			ANOVA table		
	Control	CDDP	Pair-fed	Control	CDDP	Pair-fed	Zn	Treat	Zn × T
Zn, μmol/kg[2]	1659 ± 19	1916 ± 65	1650 ± 25	2570 ± 105	3064 ± 83	2931 ± 143			
Cu, μmol/kg	422 ± 26	310 ± 6	382 ± 13	430 ± 36	387 ± 12	404 ± 6			
Fe, μmol/kg	3402 ± 117	3976 ± 198	3783 ± 201	3173 ± 107	3847 ± 142	3501 ± 75	0.087[3]	0.001	NS
Pt, μmol/kg	—	753 ± 46	—	—	851 ± 36	—	NS	—	—
MT, μmol/kg	18 ± 2	22 ± 0.9	18 ± 1.5	48 ± 6	49 ± 4	50 ± 4	0.001	NS	NS
ACE	121 ± 5	143 ± 9	114 ± 4	186 ± 4	205 ± 11	198 ± 7	0.001	0.013	NS
AP	7.9 ± 0.5	3.6 ± 0.2	6.9 ± 0.4	7.1 ± 0.3	3.0 ± 0.2	6.6 ± 0.4	0.059[3]	0.001	NS
GGT	61.6 ± 2.4	36.7 ± 3	68.3 ± 4	66.6 ± 5	34.2 ± 3.0	66.9 ± 4	NS	0.001	NS
Ln-transformed data									
Zn	7.4 ± 0.01	7.6 ± 0.03	7.4 ± 0.02	7.8 ± 0.04	8.0 ± 0.03	8.0 ± 0.05	0.001	0.001	NS
Cu	6.0 ± 0.06	5.7 ± 0.02	5.9 ± 0.03	6.0 ± 0.08	5.6 ± 0.03	6.0 ± 0.02	0.020	0.001	0.075[3]

Pairwise Comparisons of Means for Factor Treatment[4]

	Zn	Cu	Fe	ACE	AP	GGT
Controls	a	a	a	a	a	a
CDDP	b	b	b	b	b	b
Pair-fed	c	a	b	a	c	a

[1]Values represent means ± SEM of 7–10 replicates. MT, metallothionein; ACE, angiotensin converting enzyme, pkat/mg protein; AP, alkaline phosphatase, nkat/mg protein; GGT, gamma-glutamyl transferase, nkat/mg protein; NS, not significant.
[2]On a dry-weight basis.
[3]To show that the probability of a difference was close to 95%.
[4]Treatments with different letters within variables are significantly different from each other with a P value of 0.05 or less.

Discussion

Previous studies by Pogach et al.[4] showed that treating adult rats with CDDP resulted in detrimental effects on the pituitary-testicular axis. The effects were manifested by decreased serum concentrations of follicle-stimulating hormone, luteinizing hormone, and testosterone. Other effects included lower concentrations of testosterone and androgen binding protein (ABP) in the testes. They also showed that treating these rats with large intraperitoneal doses of Zn before CDDP administration lessened the effects of the drug. They found that the testicular concentration of ABP was not reduced as greatly in the Zn-treated rats as it was in those not treated. Previous studies by Reeves et al.[8,10,14] showed that changes in various enzyme activities in the kidney and failure to adequately clear urea and creatinine were not reversed by prior treatment with Zn. The present study was done to determine the effects of Zn pretreatment on selected enzyme activities and trace element concentrations in testes and epididymides of rats. These findings were compared with those found in the kidney. A second control group was introduced that would control for the anorexic effects of CDDP treatment.

Some of our findings on the effects of CDDP on testis parameters we measured corroborated those of Pogach et al.,[4] but others did not. Pogach observed that 2 mg CDDP/kg BW over a five-day period significantly reduced testicular and epididymal weight of 400 g rats. We gave 3 mg CDDP/kg BW over 3 days and did not observe a reduction in weights of corresponding organs of 200 g rats. The rats of Pogach et al. lost about 20% of their body weight while ours only lost 10%. When Pogach et al. treated rats with CDDP, the testosterone concentrations in the serum and testis were reduced to only 20% of the controls. In our study, CDDP treatment reduced the serum concentration of testosterone to about 10% of the controls. However, in the testis it was reduced to 30% of the control group. Pogach et al.[4] also observed that intraperitoneal injections of Zn (2 mg Zn as the acetate/kg BW/day) for 5 days prior to CDDP treatment significantly reduced the concentration of testosterone in the testis but not in serum. We found that Zn treatment (90 mg Zn as the carbonate in an oil emulsion/kg BW subcutaneously every other day) for 7 days prior to CDDP treatment tended to lower testosterone in serum but because of large variation, there were no significant differences. In the testis, Zn treatment reduced the testosterone concentration to only 80% of the control values. Although our method of Zn treatment was much more robust than Pogach et al.,[4] the effect on testis testosterone was much less.

We observed that the epididymal sperm count was depressed in rats treated with high subcutaneous doses of Zn. This might suggest that the number of sperm released from the testes was reduced by high Zn or that the high Zn killed the sperm already in the epididymal duct. Neither of these suggestions are defensible, however, because the concentration of Zn in the testis was not affected by Zn treatment and in the epididymis the Zn concentration was lower than control values. The circulating concentration of Zn, however, was 3–4 times higher than control values. An unknown factor detrimental to sperm might be released under these conditions.

In previous reports,[8,10,14] we showed that both Zn and CDDP treatments affect the trace mineral concentrations of kidney tissue. In the present study, we observed changes in trace mineral concentrations of the testes and epididymides and compared them with changes in the kidney. Because the kidney is the primary route of excretion of platinum, it was expected that the effects of CDDP on the kidney would be more noticeable than effects on other organs. This expectation was borne out with results for testis and epididymides. The concentration of Zn in the kidneys of rats treated with CDDP was higher and Cu significantly lower than in the control or pair-fed groups whether treated with Zn or not. However, there were no effects of CDDP treatment on Zn and Cu concentrations in the testis or epididymis.

Zn treatment itself affected the concentrations of Cu and Zn in kidney and epididymis but not the testis. There was a near significant ($P < 0.06$) depression of Zn concentration in the epididymis of rats treated with Zn. This is in contrast with the kidney, where there was a highly significant ($P < 0.001$) elevation of Zn. Zinc treatment caused a small but significant increase in the concentrations of Cu in the kidney, but at the same time caused epididymal Cu to be significantly depressed. In previous studies, Zn treatment was inclined to decrease Fe concentration in the kidney. In this experiment, however, there was a highly significant depression of Fe in the kidneys of rats that had been treated with Zn compared with those not treated. Zn treatment alone caused a significant depression of Fe in the testis but not in the epididymis.

Earlier we had shown that CDDP treatment depressed some Zn-dependent and non–Zn-dependent enzyme activities in the kidney and enhanced others.[14] The present experiments confirm these observations. Alkaline phosphatase and GGT activities were lower in the kidneys of rats treated with CDDP. Angiotensin-converting enzyme activity, on the other hand, was significantly elevated in these rats. By contrast, all the enzymes studied in the testis and epididymis were unaffected by CDDP treatment. This is an important observation with regard to the absence of an effect on ACE activity. Some investigators have provided evidence that most of the ACE activity is located in the germinal cells.[12] Others believe that it resides in the Leydig cells.[23] In either case, because CDDP treatment affects both cell types, we would have expected the activity of ACE to be reduced; it was not.

Some observations that had not been made before concern the effects of Zn treatment on enzyme activities. Unexpectedly, the activity of ACE was on the average 56% higher in kidneys of rats treated with Zn than in those not treated. There is no obvious explanation for this phenomenon. In the other tissues, the

Zn-dependent enzyme activities were depressed by Zn treatment while GGT activity was unaffected. Angiotensin-converting enzyme activity of the epididymis is located in both the sperm and in the epididymal tissue itself.[24] The observation that there were fewer sperm in the epididymis of rats treated with Zn than in those not treated suggests that this might be the cause of the lower ACE activity in this tissue. However, in the present experiment, the sperm were removed from the epididymis before ACE activity was determined in the remaining tissue. This suggests that Zn was directly affecting the tissue portion of epididymal ACE activity. Sperm ACE activity was not determined.

Induction of MT and subsequent binding of Pt was proposed as the mechanism responsible for alleviation of the toxic effects of CDDP on kidney of Zn- or Bi-treated mice.[6] This general hypothesis is not supported by this and previous studies,[6,8,10] which showed that although MT concentration in the kidneys of Zn-treated rats receiving CDDP was 2–4 times that of rats not treated with Zn, there was no protection from CDDP toxicity. Pogach et al.[4] determined the effects of high Zn doses on CDDP toxicity in the testes. Although they found that treatment of rats with Zn prior to CDDP reduced some of the testicular effects, it is unlikely that MT was the causative agent. As the present study showed, Zn does not effectively induce MT synthesis and accumulation in the testis of rats.

In summary, contrary to the suggestions that high parenteral Zn treatment protects against kidney and testicular damage, this study shows no such positive effects. However, it does demonstrate that Zn and CDDP treatment have myriad effects on tissue concentrations of Zn, Cu, and Fe and on the activities of selected membrane-bound enzymes in these organs. Although some of the effects were significant, the differences were very small. However, considering that the treatment regimens in this experiment were more or less acute, chronic treatment over a much longer period might have exaggerated many of the small differences. Humans treated with CDDP for cancer would more than likely receive doses for long periods.

Acknowledgments

The authors acknowledge Brenda Skinner for her technical assistance, Denice Schafer and her staff for animal care, and Marie Swenson for manuscript preparation and management.

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