Effect of *cis*-diamminedichloroplatinum (II) on metallothionein induction and trace element metabolism in rats fed different amounts of dietary zinc

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Recent studies have suggested that the induction of metallothionein synthesis in kidneys of mice by the acute administration of bismuth and other trace elements might protect against cis-*diamminedichloroplatinum (II) nephrotoxicity. The present study was designed to determine the effects of dietary zinc and* cis-*diamminedichloroplatinum (II) on the induction of liver and kidney metallothionein and its subsequent effect on nephrotoxicity and trace element metabolism in rats. Male rats were fed diets containing 5, 20, 80, or 320 mg zinc/kg diet for 3 weeks. Each dietary group was subdivided into 3 groups. In one group, each rat received an i.p. injection of 7.5 mg* cis-*diamminedichloroplatinum (II)/kg b.w. All other rats received saline. During the next three days a second group of rats was pair-fed to the* cis-*diamminedichloroplatinum (II) injected group. A third group received no treatment and was allowed to eat* ad libitum. *Results showed that when dietary zinc was increased from 5 mg/kg diet to higher amounts, kidney metallothionein concentration increased twofold.* cis-*diamminedichloroplatinum (II) treatment increased kidney metallothionein even further, but elevated metallothionein gave no protection from the toxic effects of the drug. Serum copper concentration and ceruloplasmin activity were significantly lower with higher concentrations of dietary zinc, which indicated that these rats were mildly copper-deficient. There was a small but significant depression of superoxide dismutase activity and a highly significant increase in thiobarbituric acid reactive substances in kidneys of rats treated with* cis-*diamminedichloroplatinum (II) compared to either pair-fed or* ad libitum *controls. This supports the hypothesis that part of the mechanism for* cis-*diamminedichloroplatinum (II)–induced toxicity might be caused by free-radical generation. However, the data do not support the hypothesis that metallothionein induction protects the kidney from* cis-*diamminedichloroplatinum (II) toxicity.*

Keywords: zinc; copper; iron; metallothionein; superoxide dismutase; lipid peroxidation

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-Pt) is an effective drug in the treatment of a variety of solid tumors. However, there are dose-limiting nephrotoxic side-effects with *cis*-Pt. Numerous reports have demonstrated that the induction of metallothionein (MT) in the kidney by the acute administration of bismuth and other trace elements such as copper, zinc, or selenium will protect against *cis*-Pt toxicity in mice.[1-4] It was shown recently that low copper status in rats enhanced toxic side effects of *cis*-Pt.[5] Because zinc is relatively non-toxic and a good inducer of MT, we designed an experiment to determine the effects of chronic feeding of various concentrations of dietary zinc on kidney MT induction and the subsequent effects on *cis*-Pt nephrotoxicity in rats.

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Materials and methods

Animals and design

Eighty-four male Sprague-Dawley (Harlan, Madison, WI,* USA) male rats, weighing 88 ± 3 g were divided into 4 dietary groups. They were fed the basal diet (*Table 1*) containing 5, 20, 80, or 320 mg of zinc per kg for 3 weeks. At the end of this period, each dietary group was divided into 3 groups of 7 rats each. One of these groups received i.p. injections of 7.5 mg *cis*-Pt per kg b.w. while the remaining 2 groups received saline injections. Over the next 3 days one of the saline injected groups was pair-fed to the *cis*-Pt–treated group. The third group was fed *ad libitum*.

Three days after *cis*-Pt treatment, rats in all groups were anesthetized (50 mg pentobarbital sodium/kg b.w.) and blood and tissues collected. Both kidneys and a portion of liver were excised and frozen immediately in liquid nitrogen. These tissues were stored at −80°C until analyzed for trace elements and MT. Total superoxide dismutase (SOD) activity and thiobarbituric acid (TBA) reactive matter were also estimated in kidney tissues. Serum was collected from clotted blood and analyzed for trace element content, urea, and ceruloplasmin.

Analytical methods

For trace element analysis, tissues were ashed in acid-washed fused quartz crucibles in a muffle furnace at 500°C for 48 hours and the ash dissolved in 0.1 N HCl. Samples were analyzed for zinc, copper, iron, and platinum by Inductively Coupled Argon Plasma Emission Spectroscopy (ICAP) (Perkin-Elmer, Model 6500, Norwalk, CT, USA). Serum samples were treated with 10% sulfosalicylic acid (SA) (serum: SA:H_2O, 0.5:0.25:0.25 ml) and zinc, copper, and iron determined by ICAP. Serum urea was determined by procedures developed by Sigma Chemical Co. (Kit# 535-B). Kidney and liver MT was determined by the ^{109}Cd/hemoglobin affinity assay of Eaton and Toal.[6] Serum ceruloplasmin activity was determined by the method of Schosinsky *et al.*[7] Total kidney SOD activity was determined by the method of Marklund and Marklund.[8] Concentrations of TBA-reactive matter in kidney tissues were estimated by the method of Asakawa and Matsushita.[9] Kidney tissue was homogenized, 1:10 (w/v), in ice-cold 1.15% KCl in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 175,000*g* for 10 minutes at 4°C and the supernatant used for the TBA-reactive matter estimation. Malondialdehyde tetramethyl acetal (99%, Eastman Kodak, Rochester, NY, USA) was hydrolyzed in acid and used as the standard. Thus the tissue concentration of TBA-reactive matter was expressed as malondialdehyde equivalents (MDAeq).

Table 1 Composition of the basal diet

Ingredients	g/kg
Corn starch	330
Glucose hydrate	300
Dried egg white[1]	175
Corn oil[2]	50
Soybean oil[3]	50
Modified AIN-76A mineral premix[4]	35
Cellulose[5]	30
AIN-76A vitamin premix[6]	10
Choline premix[7]	10
Biotin premix[8]	10

[1] Teklad, Madison, WI, Cat. #160230.
[2] Mazola Oil, Best Foods CPC International, Inc., Englewood Cliffs, NJ, USA.
[3] Crisco Oil, Procter & Gamble, Cincinnati, OH, USA.
[4] Mineral Premix (Teklad, Madison, WI Cat. #TD87282) provided the following ingredients in g per kg of diet: $CaHPO_4$, 17.5; $K_3C_6H_5O_7 \cdot H_2O$ (Potassium citrate), 7.7; K_2SO_4, 1.82; NaCl, 1.092; MgO, 0.84; Ferric citrate (17% Fe), 0.21; $MnCO_3$, 0.1225; $CrK(SO_4)_2 \cdot 12H_2O$, 0.01925; $CuCo_3$, 0.0105; KI, 0.00035; $Na_2SeO_3 \cdot 5H_2O$, 0.00035. The mineral mix provided 0.43 g of Na per kg of diet and dried egg white provided approximately 1.7 g Na per kg of diet, for a total Na content of 2.13 g per kg diet. $ZnCO_3$ was used as the source of Zn in the supplemented diets.
[5] Teklad, Madison, WI, USA, Cat. #160390.
[6] Teklad, Madison, WI, USA, Cat. #40077.
[7] 250 g of choline bitartrate per kg in finely powdered dextrose.
[8] 80 mg of d-biotin per kg in finely powdered dextrose.

Statistical methods

Results were evaluated by analysis of variance as a 3 × 4 factorial using the Crunch Statistical Package (Crunch Software Corp., Oakland, CA, USA). Differences between means were determined by the method of Scheffé.[10]

Results

Elevation of urea concentration in serum is considered a good indicator of nephrotoxicity in *cis*-Pt–treated rats. In the present study all dietary zinc groups treated with *cis*-Pt had markedly higher serum urea than those not treated or those pair-fed (*Figure 1*). There seemed to be a dose response in that serum urea concentrations became progressively higher as dietary zinc increased from 5 to 320 mg/kg of diet. However, because of high variability no significant dietary zinc effect was found. Pair-feeding, compared to no treatment, had no effect on serum urea.

Figure 2 shows effects on body weight change in rats during the 3 days after a single i.p. injection of 7.5 mg *cis*-Pt/kg b.w. This change in weight was mainly the result of a reduction in food consumption (data not shown). Body weights of pair-fed rats were also reduced during the same period but not as much as *cis*-Pt–treated rats. Body weights of non-treated rats increased.

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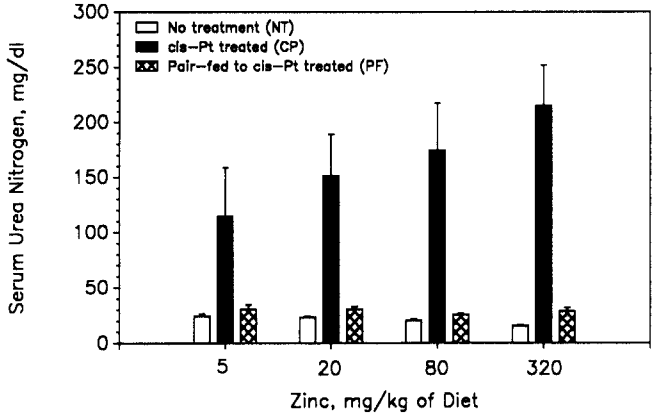


Figure 1 Bars represent means ± SEM for 7 replicates. An ANOVA showed that the only significant difference was due to treatment ($P < 0.001$). Scheffé's comparisons for this effect showed the following differences: NT, a; CP, b; PF, a. Different treatments with the same superscript letter are not significantly different from each other.

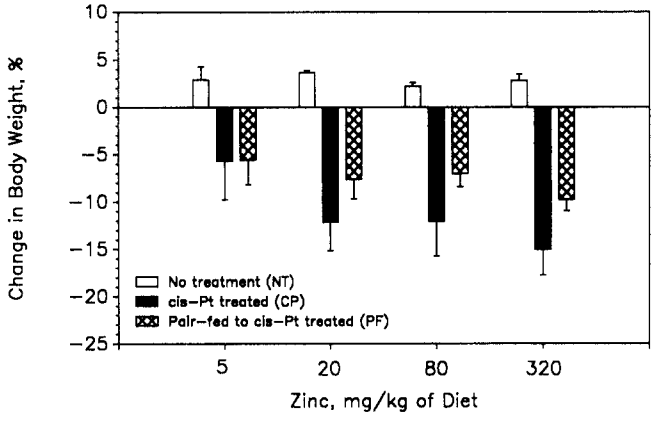


Figure 2 Bars represent means ± SEM for 7 replicates. An ANOVA showed a highly significant treatment ($P < 0.001$) but no dietary zinc effect. Scheffé's comparisons for the treatment effect showed the following differences: NT, a; CP, b; PF, b. Different treatments with the same superscript letter are not significantly different from each other.

Increasing dietary zinc from 5 to 20 mg/kg caused nearly a two-fold increase in the amount of MT in both kidney and liver of the group not receiving a treatment (*Figures 3* and *4*). Increasing dietary zinc further, from 80 to 320 mg/kg, had no effect on MT concentration. When rats were treated with *cis*-Pt there was a significant elevation of kidney MT over that observed in the non-treated group (*Figure 3*). This occurred with each increase in dietary zinc. Pair-feeding itself caused only a slight elevation of kidney MT in the 5, 20, and 80 mg Zn/kg groups. However, in the 320 mg group there was a 2.3-fold increase in pair-fed rats compared to those with no treatment. In this dietary group, pair-fed rats were not significantly different from *cis*-Pt treated rats.

cis-Pt treatment and pair-feeding affected liver MT differently than kidney MT (*Figure 4*). *cis*-Pt treatment stimulated liver MT production in the 20, 80 and 320 mg Zn/kg dietary groups, relative to the non-treated group, but so did pair-feeding. *cis*-Pt had no effect on MT in the 5 mg/kg zinc group but it had a marked effect in the 320 mg/kg group (a 7.5-gold stimulation). Pair-feeding stimulated MT production in this diet group as well but only about one-half that of cis-Pt treated rats.

Because there is an interrelationship in the metabolism of zinc, copper, and iron, the concentrations of all three of these elements were determined in serum, kidney, and liver. As expected there was a significant effect of changing dietary intake of zinc on the concentration of zinc in serum (*Table 2*). There was as much as a two-fold decrease in serum zinc in rats fed 5 mg zinc/kg diet compared to the normal 20 mg/kg. Moreover, when the highest amount of dietary zinc was fed

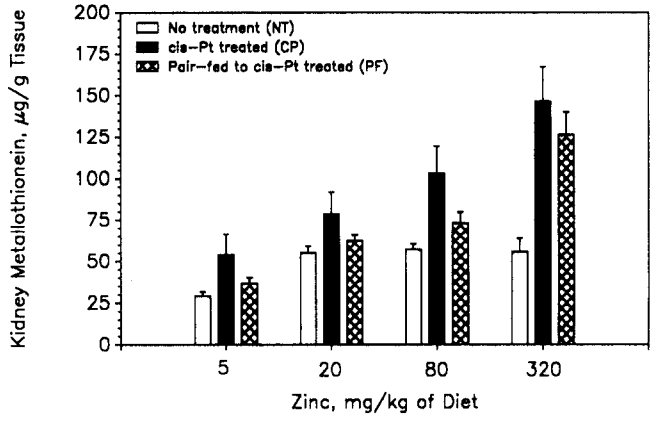
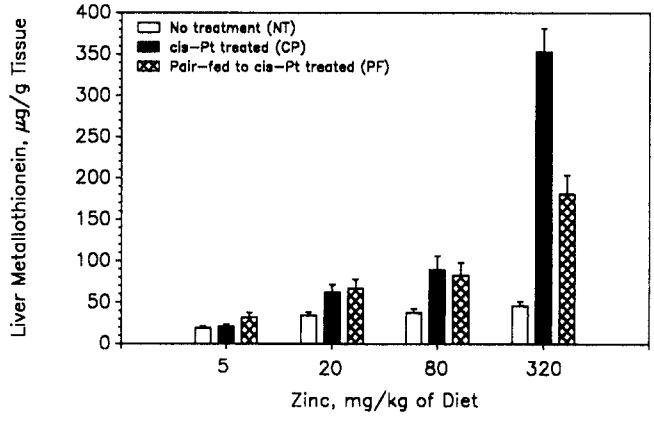


Figure 3 Bars represent means ± SEM for 7 replicates. An ANOVA showed a highly significant treatment ($P < 0.001$) and dietary zinc effects ($P < 0.001$) but no interaction. Scheffé's comparisons for the treatment effect showed the following differences: NT, a; CP, b; PF, c. Scheffé's comparisons for the zinc effect showed the following differences: 5, a; 20, b; 80, b; 320, c. Different treatments and zinc levels with the same superscript letter are not significantly different from each other.



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(320 mg/kg), serum zinc concentration was significantly elevated even further compared to the lower dietary zinc intakes. There was no overall effect of *cis*-Pt or pair-feeding on serum zinc.

There was a slight treatment effect and a highly significant zinc effect on the concentration of copper in serum, the pair-fed group was higher than the non-treated group (*Table 2*). *cis*-Pt treatment had no effect on serum copper concentration. Feeding various concentrations of dietary zinc significantly affected serum copper concentrations. When rats consumed diets containing 320 mg zinc/kg, the concentration of serum copper was significantly lower than in rats consuming any of the other dietary levels of zinc.

There was a small effect of treatment and a highly statistically significant effect of zinc on iron in serum (*Table 2*). Overall, serum iron was higher in *cis*-Pt–treated than pair-fed or non-treated rats. The concentration of iron in serum tended to be higher when rats consumed low zinc diets but only the 5 and 80 mg/kg groups were significantly different.

Both treatment and dietary zinc significantly affected the concentration of zinc in kidney (*Table 3*).

Table 2 Effect of *cis*-Pt treatment and pair-feeding on the concentration of zinc, copper, and iron in serum of rats fed different amounts of dietary zinc[1]

Dietary Zn mg/kg	Treatment: No Treatment (NT)	*cis*-Pt (CP)	Pair-fed (PF)
	Zinc, μg/ml		
5[a2]	0.68 ± 0.04[x4]	0.79 ± 0.09[x]	1.02 ± 0.04[x]
20[b]	1.49 ± 0.05[y]	1.35 ± 0.08[xy]	1.37 ± 0.05[x]
80[b]	1.55 ± 0.07[y]	1.76 ± 0.10[yz]	1.47 ± 0.05[xy]
320[c]	2.42 ± 0.16[a,z]	2.16 ± 0.11[ab,z]	1.82 ± 0.11[b,y]
	NT[3]	CP	PF
	Copper, μg/ml		
5[a]	0.74 ± 0.04	0.75 ± 0.02	0.77 ± 0.04
20[a]	0.77 ± 0.03	0.81 ± 0.03	0.82 ± 0.02
80[a]	0.68 ± 0.02	0.84 ± 0.05	0.80 ± 0.05
320[b]	0.52 ± 0.11	0.63 ± 0.08	0.68 ± 0.03
	NT[a]	CP[ab]	PF[b]
	Iron, μg/ml		
5[a]	2.07 ± 0.12	2.06 ± 0.09	1.86 ± 0.08
20[ab]	1.83 ± 0.05	1.94 ± 0.11	1.61 ± 0.09
80[b]	1.59 ± 0.08	1.90 ± 0.12	1.74 ± 0.11
320[ab]	1.66 ± 0.07	2.10 ± 0.21	1.69 ± 0.09
	NT[a]	CP[b]	PF[a]

	ANOVA Table: Serum Zn	Serum Cu	Serum Fe
Dietary Zn	0.001	0.001	0.030
Treatment	NS	0.020	0.001
Zn × T	0.001	NS	NS

[1] Values are means ± SEM for 7 replicates.
[2] Scheffé's comparisons for the zinc factor.
[3] Scheffé's comparisons for the treatment factor.
[4] Scheffé's comparisons for the interaction between zinc and treatment. a,b denotes differences between means across treatments within an individual zinc level. x,y,z denotes differences between means across zinc levels within an individual treatment. Zinc or treatment levels with the same superscript letter are not significantly different from each other.

Table 3 Effect of *cis*-Pt treatment and pair-feeding on the concentration of zinc, copper, iron, and platinum in kidney of rats fed different amounts of dietary zinc[1]

Dietary Zn mg/kg	Treatment: No Treatment (NT)	*cis*-Pt (CP)	Pair-fed (PF)
	Zinc, μg/g Dry Tissue		
5[a2]	86.2 ± 3.3	105.9 ± 6.9	93.5 ± 3.2
20[b]	95.8 ± 4.4	121.9 ± 5.4	103.6 ± 4.4
80[b]	97.8 ± 4.1	111.9 ± 5.3	113.6 ± 2.5
320[c]	133.3 ± 3.3	142.9 ± 8.3	142.3 ± 4.7
	NT[a3]	CP[b]	PF[b]
	Copper, μg/g Dry Tissue		
5[a]	29.9 ± 1.7	26.5 ± 1.4	22.8 ± 1.3
20[a]	29.1 ± 0.5	23.2 ± 3.0	23.3 ± 2.3
80[b]	22.6 ± 2.5	18.9 ± 1.0	22.3 ± 1.2
320[b]	19.4 ± 0.9	17.8 ± 0.3	20.2 ± 0.9
	NT[a]	CP[b]	PF[b]
	Iron, μg/g Dry Tissue		
5	198.2 ± 10.7	237.0 ± 21.4	202.9 ± 5.3
20	190.2 ± 10.0	205.8 ± 14.4	202.0 ± 11.5
80	181.7 ± 3.9	202.2 ± 11.8	192.8 ± 2.6
320	180.7 ± 5.7	194.7 ± 10.2	206.1 ± 7.2
	NT[a]	CP[b]	PF[ab]
	Platinum, μg/g Dry Tissue		
5	0.0	8.2 ± 4.1	0.0
20	0.0	15.5 ± 5.0	0.0
80	0.0	15.0 ± 2.5	0.0
320	0.0	25.1 ± 5.5	0.0

	ANOVA Table: Kidney Zn	Kidney Cu	Kidney Fe
Dietary Zn	0.001	0.001	NS
Treatment	0.001	0.005	0.020
Zn × T	NS	NS	NS

[1] Values are the means ± SEM for 7 replicates.
[2] Scheffé's comparisons for the zinc factor.
[3] Scheffé's comparisons for treatment factor. Zinc or treatment levels with the same superscript letter are not significantly different from each other.

Both *cis*-Pt–treated and pair-fed groups had higher kidney zinc than the non-treated group. Kidney zinc in the *cis*-Pt–treated group was not significantly different from the pair-fed group. As dietary zinc was elevated there was a progressive increase in kidney zinc. Kidneys from rats with the higher zinc intake had 1.5 times as much zinc as those given the lowest zinc intake. Treatment significantly affected kidney copper as well, however, in the opposite direction than that observed with zinc. Compared to the non-treated group, *cis*-Pt treatment and pair-feeding significantly depressed kidney copper. On the other hand, *cis*-Pt treatment and pair-feeding tended to increase kidney iron, which was significant only for the *cis*-Pt–treated and non-treated groups.

The platinum concentration in kidney varied with dietary zinc (*Table 3*). There was a tendency for platinum to be depressed in the kidneys of rats consuming the lowest amount of zinc. However, due to high variability there was no significant difference between groups as determined by a one-way analysis of

variance. Kidney platinum concentration followed the elevated concentration of kidney metallothionein as dietary zinc was increased ($R = 0.77, P < 0.001$). This suggests that platinum might be bonded to the low molecular weight ligand.

As in kidney, both treatment and dietary zinc significantly affected the concentration of zinc in the liver (*Table 4*). Both *cis*-Pt–treated and pair-fed groups had higher liver zinc than the non-treated group. Increasing dietary zinc from 5 to 320 mg/kg increased liver zinc about 1.7-fold in the *cis*-Pt–treated group and to a lesser amount in the other two groups. Liver copper concentrations were lower in *cis*-Pt–treated rats than in pair-fed rats but not in the non-treated rats. Liver copper in pair-fed rats was significantly higher than non-treated rats. The major effect of increasing dietary zinc was a depression in the amount of liver copper.

Table 4 Effect of *cis*-Pt treatment and pair-feeding on the concentration of zinc, copper, iron, and platinum in liver of rats fed different amounts of dietary zinc[1]

Dietary Zn mg/kg	Treatment: No Treatment (NT)	*cis*-Pt (CP)	Pair-fed (PF)
	Zinc, μg/g Dry Tissue		
5[a2]	84.9 ± 2.6	88.7 ±5.3[x]	96.3 ± 3.7
20[b]	96.7 ± 3.0	109.8 ± 5.6[x]	119.8 ± 9.1
80[ab]	94.4 ± 1.2	107.5 ± 4.0[x]	110.0 ± 4.1
320[c]	106.6 ± 4.4[a4] NT[a3]	152.6 ± 12.0[b,y] CP[b]	122.8 ± 10.8[ab] PF[b]
	Copper, μg/g Dry Tissue		
5[a]	10.7 ± 0.3	11.6 ± 0.5	13.7 ± 0.5
20[a]	11.3 ± 0.3	12.2 ± 0.5	14.5 ± 1.1
80[ab]	10.6 ± 0.3	11.9 ± 0.5	12.4 ± 0.5
320[c]	10.2 ± 0.3 NT[a]	10.3 ± 0.5 CP[a]	11.3 ± 0.8 PF[b]
	Iron, μg/g Dry Tissue		
5[a]	346.5 ± 41.4	362.2 ± 27.8	441.4 ± 32.9
20[ab]	292.2 ± 14.9	362.7 ± 34.3	402.5 ± 30.8
80[ab]	310.2 ± 14.7	336.4 ± 19.4	380.5 ± 17.7
320[b]	265.4 ± 8.8 NT[a]	288.9 ± 25.4 CP[a]	346.6 ± 31.9 PF[b]
	Platinum, μg/g Dry Tissue		
5	0.0	4.6 ± 1.8	0.0
20	0.0	4.8 ± 1.4	0.0
80	0.0	4.9 ± 1.0	0.0
320	0.0	4.3 ± 1.3	0.0

	ANOVA Table: Liver Zn	Liver Cu	Liver Fe
Dietary Zn	0.001	0.001	0.001
Treatment	0.001	0.001	0.004
Zn × T	0.020	NS	NS

[1] Values are means ± SEM for 7 replicates.
[2] Scheffé's comparisons for the zinc factor.
[3] Scheffé's comparisons for the treatment factor.
[4] Scheffé's comparisons for the interaction between zinc and treatment. a,b denotes differences between means across treatments within an individual zinc level. x,y,z denotes differences between means across zinc levels within an individual treatment. Zinc or treatment levels with the same superscript letter are not significantly different from each other.

Table 5 Effect of *cis*-Pt treatment and pair-feeding on the activity of ceruloplasmin in serum of rats fed different amounts of dietary zinc[1]

Dietary Zn mg/kg	Treatment: No Treatment (NT)	*cis*-Pt (CP)	Pair-fed (PF)
	Ceruloplasmin, Units/L		
5[a2]	96.5 ± 4.5	100.7 ± 4.1	103.8 ± 5.3
20[a]	101.6 ± 4.1	112.5 ± 4.9	108.4 ± 4.2
80[a]	86.2 ± 4.5	104.9 ± 9.0	106.2 ± 7.8
320[b]	57.9 ± 14.4 NT[a3]	83.6 ± 14.8 CP[b]	87.8 ± 4.2 PF[b]

	ANOVA Table: Ceruloplasmin
Dietary Zn	0.001
Treatment	0.003
Zn × T	NS

[1] Values are the means ± SEM for 7 replicates.
[2] Scheffé's comparisons for the zinc factor.
[3] Scheffé's comparisons for treatment factor. Zinc or treatment levels with the same superscript letter are not significantly different from each other.

Effects of treatment and dietary zinc on liver iron were similar to those on liver copper. *cis*-Pt treatment elevated liver iron while pair-feeding elevated it even more, so that there was no significant difference between these two groups. Increasing dietary zinc lowered liver iron but only groups fed 5 or 320 mg zinc/kg diets were significantly different.

Even though liver metallothionein increased as dietary zinc increased, there was a constant concentration of platinum in liver across all dietary levels of zinc. An increase in metallothionein and platinum in kidney suggests that platinum is bonded to metallothionein. However, in liver, even with a marked increase in metallothionein with the 320 zinc/kg diet, there was not an increase in the concentration of liver platinum. This suggests that the low molecular weight ligand did not bond platinum in this tissue.

Effects of feeding high zinc on serum copper concentration indicated that the rats might have had a marginal to low copper status. Serum ceruloplasmin values for these rats support this possibility (*Table 5*). Ceruloplasmin values for rats fed the 320 mg zinc/kg diet were significantly lower than those from rats fed the other three concentrations of dietary zinc. Serum ceruloplasmin values for both the *cis*-Pt–treated and pair-fed groups were significantly higher than the non-treated group.

Superoxide dismutase activity was significantly depressed in the *cis*-Pt–treated group (*Table 6*). The reduction, however, was only about 7% below the control value. One might conclude that such a small reduction in SOD would be insufficient to make a significant physiological change in the concentration of superoxide that might be generated during *cis*-Pt treatment. Dietary zinc had no effect on SOD activity.

Both treatment and dietary zinc had a significant effect on the estimated concentration of TBA-reactive

Table 6 Effect of *cis*-Pt treatment and pair-feeding on the activity of superoxide dismutase (SOD) and the concentration of TBA-reactive lipid (MDAeq) in kidneys of rats fed different amounts of dietary zinc[1]

Dietary Zn mg/kg	Treatment		
	No Treatment (NT)	*cis*-Pt (CP)	Pair-fed (PF)
	SOD Activity, Units/mg Protein		
5	11.9 ± 0.5	11.9 ± 0.2	12.5 ± 0.3
20	12.6 ± 0.3	12.2 ± 0.6	12.5 ± 0.3
80	12.6 ± 0.3	11.1 ± 0.5	11.8 ± 0.7
320	12.3 ± 0.1	10.7 ± 0.4	12.6 ± 0.4
	NT[a3]	CP[b]	PF[a]
	MDAeq, nmol/mg Protein		
5[a2]	1.51 ± 0.06	1.63 ± 0.16	1.44 ± 0.93
20[ab]	1.28 ± 0.08	1.48 ± 0.07	1.26 ± 0.06
80[b]	0.97 ± 0.12	1.62 ± 0.13	1.23 ± 0.12
320[b]	0.97 ± 0.09	1.31 ± 0.06	1.27 ± 0.10
	NT[a]	CP[b]	PF[a]

	ANOVA Table	
	SOD Acitivty	MDAeq
Dietary Zn	NS	0.001
Treatment	0.005	0.001
Zn × T	NS	NS

[1] Values are the means ± SEM for 7 replicates.
[2] Scheffé's comparisons for the zinc factor.
[3] Scheffé's comparisons for treatment factor. Zinc or treatment levels with the same superscript letter are not significantly different from each other.

substances in kidney (*Table 6*). The estimated MDAeq concentration was significantly elevated by *cis*-Pt treatment compared to either control group. The estimated MDAeq was significantly elevated when dietary zinc was low compared to when it was high. These effects were most notable in the group receiving no treatment. Effects of zinc were diminished in both the *cis*-Pt–treated and the pair-fed groups compared to the non-treated groups.

Discussion

Imura *et al.*[1-4] have suggested repeatedly that the induction of MT in the kidney by trace elements, especially bismuth, will protect mice from lethal doses of *cis*-Pt. Metallothionein apparently inhibits the nephrotoxic effects of the drug. Because zinc is a good inducer of MT, it was the purpose of the present study to determine if nephrotoxicity of *cis*-Pt treatment could be reversed or diminished by feeding high dietary zinc for an extended period. Rats were fed four concentrations of dietary zinc ranging from 5 to 320 mg zinc/kg of diet for four weeks. Results of this study showed that, instead of lessening the effects of *cis*-Pt on the kidney, high dietary zinc tended to make them worse; as indicated by an increase in serum urea and by an increased reduction in body weight as dietary zinc increased. However, these changes were not statistically significant. Similar concentrations of platinum in livers of all groups indicated that all rats received a similar dose of *cis*-Pt, even though kidney platinum tended to vary with dietary zinc. Although MT concentrations in kidney were increased by the highest concentrations of dietary zinc, this provided no protection from the drug.

The reason for this lack of protection could lie in the fact that feeding the diet that contained high zinc induced a mild copper deficiency. The evidence that rats fed 320 mg zinc/kg diet were copper-deficient consisted of lower serum copper concentrations and ceruloplasmin activities that were lower than those in the lower dietary zinc groups. It was shown previously[5,11] that copper deficiency, initiated by feeding rats low copper diets, enhanced the nephrotoxic effects of *cis*-Pt. Imura *et al.*[1] also showed that pretreatment of tumor cell–implanted mice with $CuSO_4$ increased survival rates to 100% when treated with *cis*-Pt. Kidney MT concentrations were not affected. Thus, in the present study, effects of an induced copper deficiency may have canceled any ameliorative effects that zinc-induced MT might have had. Therefore, the effect of trace element pretreatment on *cis*-Pt nephrotoxicity may not be totally the result of the induction of MT. Recent studies in our laboratory have demonstrated that increasing the dietary copper from 6 to 18 mg/kg of diet did not prevent *cis*-Pt–induced kidney toxicity (unpublished data).

Naganuma *et al.*[3] found a response in kidney MT synthesis to bismuth administration similar to ours with zinc. When they administered bismuth nitrate (16–67 mg/kg b.w./day) for 5 days, they found the concentation of kidney MT increased (60–210 nmol/g tissue). They did not, however, give MT values for those mice receiving both bismuth and *cis*-Pt or *cis*-Pt alone. Farnsworth *et al.*[12] did, however, find a twofold rate of increase in renal MT in mice injected with *cis*-Pt. In our experiments, the higher the dietary zinc the higher was MT induction by *cis*-Pt. Without *cis*-Pt treatment, MT induction plateaued after dietary zinc reached 20 mg/kg of diet. These studies suggest that there is a maximum to which kidney MT can be induced by elevating dietary zinc. However, the constant intake of a high amount of zinc primes the tissue for further induction of MT by other factors; in this case the side effects of a drug and the stress of pair-feeding.

Some investigators[12,13] have been unable to find an effect of *cis*-Pt treatment on trace element concentrations in kidney and liver. Others[14] have observed decreased zinc and copper concentrations in kidney. Reeves *et al.*[5] found kidney zinc to be higher in *cis*-Pt–treated rats than controls. None of these studies used to the pair-fed animal as a control. In the present study, we incorporated a group of rats that was pair-fed in relation to the group receiving the *cis*-Pt treatment. We observed a significantly higher concentration of zinc in kidneys of *cis*-Pt–treated rats when compared to nontreated rats. However, when compared to the pair-fed group there were no significant differences. This higher concentration of kidney zinc with *cis*-Pt treatment was seen primarily in rats fed the

lowest concentrations of dietary zinc. Conversely, we observed a decreased copper concentration in *cis*-Pt–treated kidneys when compared to kidneys from non-treated rats. Again, there was no significant effect when the *cis*-Pt group was compared to the pair-fed group.

Pair-feeding also had a significant effect on liver copper and iron. Concentrations of both metals were higher in the pair-fed groups that in those treated with *cis*-Pt or non-treated. Solecik *et al.*[15] observed elevated iron but not copper in pair-fed rats compared to *ad libitum*–fed controls. Conrad *et al.*[16] fasted rats for 5 days and observed an increased liver iron concentration compared to that in rats not fasted. It is evident from the present experiment and others that the pair-fed animal should be used in the study of *cis*-Pt toxicity to get meaningful and comparable results.

Mechanisms of *cis*-Pt induced toxicity are not understood but may be related to the formation of highly reactive O_2-derived species such as superoxide, hydroxyl radicals, and hydrogen peroxide. These species react with cellular membrane lipids and proteins to cause cellular damage.[17] Platinum IV was shown to stimulate the production of superoxide by phagocytes or xanthine oxidase.[18] Superoxide has been implicated in the mechanism for *cis*-Pt toxicity by McGuinness *et al.*,[19] who pretreated rats with superoxide dismutase and found that drug-induced nephrotoxicity was weakened. More recent studies implicated free radicals in *cis*-Pt toxicity when it was observed that lipid peroxidation occurred less frequently in kidney slices incubated with *cis*-Pt and antioxidants than with *cis*-Pt alone.[20] Rats pretreated with an antioxidant also had less severe symptoms of *cis*-Pt toxicity than those not treated.[21] In the present study we observed a small but significant decrease in kidney SOD activity as a result of *cis*-Pt treatment. In addition, there was a highly significant increase in the concentration of MDAeq in the kidney of these drug-treated rats. This suggests that perhaps part of the mechanism of nephrotoxicity in *cis*-Pt–treated rats is related to free radical formation.

We also observed a highly significant effect of dietary zinc on TBA-reactive matter in kidney tissue. As dietary zinc increased there was an apparent reduction in kidney lipid peroxidation, especially in the non-treated group. However, less TBA-reactive matter with higher doses of dietary zinc did not translate into less toxicity of *cis*-Pt to the kidney. Coppen *et al.* [22] studied effects of increasing the concentration in the medium zinc on the formation of free radicals in liver cells grown in primary culture. Their results showed that as the medium concentration of zinc increased, apparent lipid peroxidation was reduced. Sullivan *et al.*[23] have shown a higher concentration of *in vivo*, and a higher rate of *in vitro*, lipid peroxidation in subcellular membranes from zinc-deficient rats compared to controls. Burke and Fenton,[24] on the other hand, showed a potentiation of lipid peroxidation in microsomal membranes of zinc-deficient rats but were unable to observe significant alterations *in vivo*.

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