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Mobilization of Tissue Cadmium in Mice and Calves and Reversal of Cadmium Induced Tissue Damage in Calves by Zinc

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Our earlier studies (Lamphere et al 1984) demonstrated that simultaneous dietary Zn supplementation to calves fed Cd, significantly decreased the accumulation of Cd in liver, kidney and muscle. However, studies are lacking in evaluating the effectiveness of zinc in reducing Cd-burden in animals with pre-existing tissue Cd-load, a situation encountered in chronic Cd intoxication. This study examined the effects of oral Zn (ZnO) on tissue Cd levels in mice. N-acetylcysteine (NAC) and sodium sulfate (SS) were also used to evaluate the effects of providing organic and inorganic sources of sulfur on tissue Cd levels.

Following demonstration of reduced Cd levels in tissues of mice receiving antidotal Zn, subsequent investigation was aimed at studying the reversal of Cd-induced changes by Zn. We also examined whether Cd-induced reduction in epididymal 5 α-reductase activty could explain previously reported low levels of circulating dihydrotestosterone (DHT) following Cd treatment (Saksena et al 1977). The ability of Zn to reverse the inhibition of 5 α-reductase activity by Cd was also examined.

MATERIALS AND METHODS

Male, Charles River CD1 mice (7 to 8 per group, Kingston, NY) were given ip 5 alternate-day doses of 0.01 mmoles of Cd/kg as $CdCl_2$ (Sigma Chemical Co., St. Louis, MO) dissolved in saline. A control (no Cd) group received ip injections of saline at 4 ml/kg. Three days after the last Cd injection, one group of control (no Cd) and Cd-treated mice (pre-antidote) were killed by ether overdose for tissue collection and analyses of Cd, Zn and Cu. The remaining Cd-treated mice were treated orally for 8 or 14 days with one of the following agents: 5 ml saline/kg/day; 0.15 mmoles of Zn/kg/day given as ZnO (Sigma); 200 mg/kg/day of N-acetylcysteine (NAC, Sigma), and 150 mg/kg/day of sodium sulfate (SS, Sigma) in distilled water. Mice were killed and tissues collected for metal analysis as described earlier (Lamphere et al 1984).

To evaluate the effects of Cd on serum enzymes, clinical chemistry, and 5 α-reductase (AR), four male Angus x Hereford

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calves (118-189 kg) were given 5 alternate day sc injections of 1.12 mg/Kg of Cd as $CdCl_2$ (0.01 mmoles/Kg) as a 5% solution in normal saline. Seven days following last injection, approximately 1 g samples of liver and kidney were obtained by surgical biopsy to evaluate tissue injury by light microscopy and establish tissue Cd levels prior to the beginning of Zn treatment. On the day following biopsy, two of the four calves were allowed to remain on the basal ration and the other two were given diet mixed to contain 1000 ppm of Zn (actual Cd, Cu and Zn contents were 0.1, 7.3 and 980 ppm, respectively) as ZnO for 45 days at a level of 2% of the animal's body weight. Weekly blood samples were collected throughout the experimental period and analyzed for Cd, Cu and Zn as well as selected clinical chemistry and enzyme parameters (BUN, Creatinine, SGOT, SGPT and alkaline phosphotase) using standard methods. Calves were killed after 45 days. Samples of liver, kidney, muscle (triceps), pancreas and spleen were collected for the analysis of metals. Slices of kidney and liver were fixed for histologic examination by standard techniques. A sample of one testis was taken for analysis of Cd,Cu and Zn and another for histologic examination. Epididymides were divided into caput-, corpus-, and cauda-, and individual regions assayed for AR activity as described below. Epididymides from two additional age and weight- matched, untreated calves from the same farm were obtained to establish control AR.

To assay AR activity, epididymides held at 0-4°C were finely minced and washed free of spermatozoa with ice-cold Krebs Ringer phosphate buffer (KRPB, pH=6.9). Tissue minces were homogenized in KRPB at 10 ml/g tissue and filtered through a 93 µm mesh and stored on ice. Twenty milligrams of tissue (220 µl of homogenate) were incubated for 1 hr at 22°C with 0.05 µCi ^{3}H-testosterone and nonradioactive testosterone (T) to give $1x10^{-7}$ M T in a 1.0 ml KRPB (pH 6.9) which also contains 5×10^{-5} M NADPH. Cold carrier standards ($1x10^{-5}$M) were added and the incubation mixture extracted with 10 ml of ethyl acetate. The tubes were centrifuged for 10 minutes at 1500 rpm in a Sorvall GLC-2B centrifuge. The aqueous layer was frozen (-70°C), the organic layer collected and evaporated to dryness in air and reconstituted with chloroform:methanol (50:50, v/v). Standard T, 5 α-DHT, and 5α -androstan-3 β -diols and the organic layer (5 µl) were spotted on an activated 20cmx20cm silica gel G plate and developed in two solvent systems: first with methylene chloride:diethyl ether (80:20, v/v) and then in benzene:acetone (80:20, v/v). The plate was allowed to air-dry and then lightly sprayed with 5% phosphomolybdic acid in acetone and then dried in an oven at 100°C for 10 minutes. Spots for each radioactive lane were identified and scraped into 7 ml plastic counting vials containing scintillation cocktail (6.0 ml). Following equilibration in total darkness for at least 20 hr, vials were counted with a Beckman LS-1800 liquid scintillation counter for two successive one minute intervals. Average of duplicate cpm values was used in all calculations, according to the method of Robaire (1979). Protein in the homogenate was determined by Lowry (1951) method. The specific activity of AR was expressed as moles of 5 α-reduced androgens formed/hr/mg protein. Total activity was expressed as moles of 5α

-reduced androgens formed/hr.

Comparison of tissue Cd levels among groups of mice given various antidotes including those receiving Cd but no antidote was made by the analysis of variance. Where comparison was made only between mice receiving or not receiving antidotal Zn, student's t-test was used. Tissue Cd levels and clinical chemistry parameters between zinc- and saline-receiving calves were compared using student's t-test. Epididymal enzyme activities among control (no Cd), and Cd- treated calves with or without antidotal dietary Zn were compared using analysis of variance. A value of $P<0.05$ was accepted as significant in all comparisons.

RESULTS AND DISCUSSION

As expected, multiple Cd treatments gradually increased tissue Cd levels to 55.4 and 21.1 μg/g in liver and kidney, respectively, by 4 days after last Cd dose (pre-antidote levels). Cadmium accumulation continued, especially in the kidney, even after cessation of Cd treatment. The loss of Cd from liver, kidney and pancreas between day 8 and day 14 of antidotal period was 4, <250, and 10 times greater in zinc receiving animals compared to saline treated mice (Fig. 1, Table 1). Cadmium levels in spleen and testes actually increased 7% and 22% during the last 6 days of saline treatment whereas the levels declined 42% and 5%,

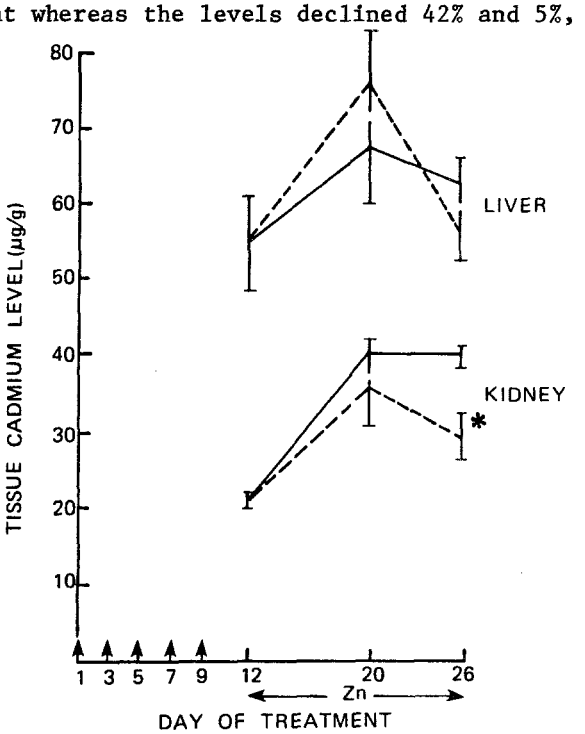

Figure 1. Reduction of tissue cadmium (mean ± SE; N=8) in mice by oral zinc given following prior cadmium loading. Solid line represents Cd-treated mice given oral zinc. Asterick indicates significant difference ($P<0.05$).

respectively, in zinc-treated animals (Table 2). Significantly ($P<0.05$) lower individual tissue Cd burdens were noted in kidney, pancreas and spleen of zinc treated mice compared to the same organ in saline-treated mice. Behari and Tandon (1980) failed to reduce kidney or liver Cd levels by Zn supplementation following Cd loading. However, they used less than a 2:1 molar ratio of Zn: Cd in their treatment. A molar ratio of 12.5:1 of Zn:Cd was needed to protect against Cd-induced testicular damage (Gunn et al. 1968). Indeed, in our studies, a 15:1 molar ratio of Zn: Cd given orally to mice for two weeks following Cd dosing resulted in a 30% reduction in kidney Cd load with no concomitant increase in Cd concentrations in other tissues. The failure of 8 day but not 14 day Zn treatment to decrease Cd levels in mouse tissues in our studies suggests that the length of treatment is also important in Zn antagonism of Cd effects and accumulation. Surprisingly, no increases in zinc levels were found in either liver or kidney of zinc receiving mice (Table 2) suggesting that tissue buildup of Zn is not a prerequisite to Cd mobilization. Significant increase (69%) in liver copper was seen in mice receiving zinc compared to those receiving saline (Table 2). Blood zinc levels in zinc-treated mice in contrast to those in tissues were 1.5 times those in Cd-saline group mice (5.3 vs 8.0 ppm). Interaction of zinc either at the level of uptake of Cd into the tissues during

Table 1. Decline in average tissue cadmium-levels between day 8 and day 14 of saline or zinc treatment in cadmium pre-treated mice

Tissue	% decline from day 8	
	Cd-Saline	Cd-Zinc
Liver	7.0	25.4
Kidney	<0.1	17.4
Pancreas	2.7	27.4
Spleen	-7.3	42.4
Testes	-21.7	5.3

the redistribution phase, or at the site of G.I. epithelium preventing the reabsorption of Cd reaching the intestines through bile or sloughed off epithelial cells is possible. Additional studies to evaluate Cd balance during the antidotal period are needed to clarify the mechanism. Also, the ability of zinc to induce metallothionein in animals whose metallothionein is already induced by Cd needs further study. Neither SS, nor NAC has any signficant effect ($P>0.05$) on tissue metal levels (data not shown).

Treatment with Cd resulted in a decrease in BUN and serum creatinine (C) levels (from 17 and 1.3 mg% to 12 and 0.6 mg%) and an increase (125% and 80%) in SGOT and SGPT in all four calves.

Table 2. Effects of 14-day zinc treatment on tissue cadmium, zinc and copper (μg/g wet weight) in cadmium-pretreated mice[a]

Tissue	Cd-Saline			Cd-Zinc		
	Cadmium	Zinc	Copper	Cadmium	Zinc	Copper
Liver	63.3+3.4	46.4+2.8	5.0+0.4	56.9+4.11	41.0+3.8	6.8+0.8[b]
Kidney	40.1+1.6	28.1+1.6	4.1+0.4	29.7+3.0[b]	24.5+0.5[b]	3.5+0.4
Pancreas	18.5+1.7	ND[c]	ND	13.5+1.2[b]	ND	ND
Spleen	6.4+0.4	ND	ND	4.6+0.5[b]	ND	ND
Testes	2.5+0.5	ND	ND	1.8+0.6	ND	ND

[a]Values are means + SE. N = 7 to 8
[b]Significantly different from Cadmium-saline group, $P<0.05$
[c]Not analyzed.

Following 45 days of zinc- or basal- diets, the levels of BUN rebounded to pre-Cd levels whereas the levels of C remained lower. The levels of SGOT and SGPT returned to normal following withdrawal of Cd with or without Zinc supplementation indicating reservible nature of hepatic damage as reported (Friberg et al 1974). Serum AP levels were significantly reduced in Cd-treated calves (Fig. 2) reflecting a functional Zn deficiency induced by Cd (Miller et al 1965). Calves exposed to Cd but kept on basal diet had only a slight recovery of AP levels to a maximum of about 80% of pre-Cd values. Serum AP in Calves receiving Zn diet, however, rebounded to somewhat greater than pre-Cd values following three weeks of Zn supplementation and remained at that level throughout the experiment. Serum AP levels in Zn-receiving calves were significantly higher ($p<0.05$) than in calves not receiving Zn throughout this period (Fig. 2). Zinc prevented Cd-induced decrease in femur AP levels (Bonner et al 1980); however, reversal following inhibition has not been shown earlier.

Light microscopic examination of biopsy tissues from Cd-treated calves revealed expected changes such as centrolobular vacuolization of hepatocytes, renal proximal tubular necrosis and mild degenerative changes in ditsal tubules. Although Cd-withdrawal resulted in marked recovery from liver and kidney damage, no differences in kidney or liver histology could be discerned in calves receiving Zn diet compared to those not receiving Zn. Although testes from both groups of calves showed moderate atrophy no discernable differences existed between the two groups (Zn and no-Zn). The levels of Cd in the liver and kidney between the two groups of calves at the beginning of basal or zinc diet following Cd treatment were not significantly different ($P>0.05$, not shown). Testicular Cd levels were significantly ($P<0.05$) reduced by high dietary zinc (Table 3).

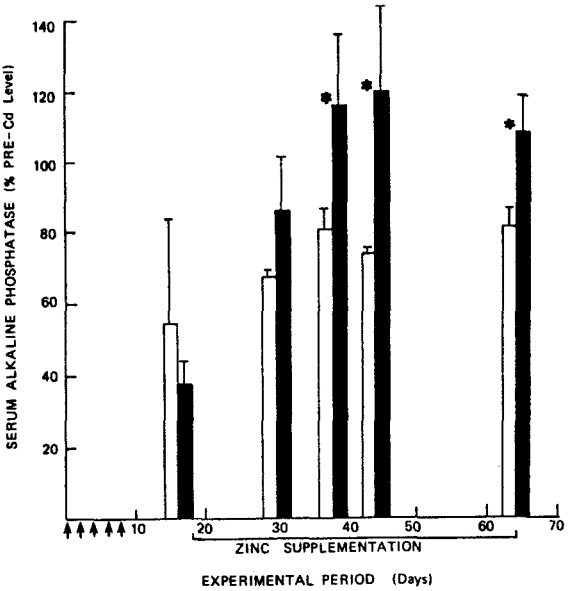

Figure 2. Reversal of cadmium-induced depression of calf serum alkaline phosphotase by Zinc. Arrows indicate cadmium injection. Light bars represent calves on basal diet and dark bars represent calves on zinc diet. Values are mean percent ± SD of alkaline phosphotase levels compared to pre-Cd values (100%) from the same calf. Astericks indicate significant differences ($P<0.05$) between groups.

Cadmium levels in all other tissues were either unchanged or declined insignificantly ($P>0.05$) following zinc therapy.

Table 3. Effect of zinc on tissue cadmium content (ug/g) in calves pretreated with cadmium.

Group	Liver	Kidney	Pancreas	Spleen	Testis
No Zinc	42.0 ± 0.7	176.0 ± 43.0	3.10 ± 1.0	2.76 ± 0.2	0.50 ± 0.03
Zinc	37.4 ± 2.3	136.8 ± 33.6	3.21 ± 0.2	2.69 ± 0.4	0.38 ± 0.01*

[a] mean ± SE bearing this superscript is significantly different ($P<0.05$) from the mean for the same tissue in the no-zinc group.

Zn levels in liver, kidney, and pancreas were 5 to 12 times higher ($P<0.05$) in Zn-receiving calves but not mice compared to those fed basal diet. This species difference in Zn accumulation may indicate different mechanisms of antagomism.

The activity of epididymal AR was about 30-35% of control in both head and tail regions and about 40% of normal in the body 45 days following cessation of Cd exposure (Fig. 3). Saksena et al (1977) observed reduced levels of T and DHT in rat blood following single subcutaneous dose of Cd. They claimed that the reduced DHT levels merely reflected the reduced levels of T. However, our studies indicate that the conversion of T to DHT is also affected by Cd. Dietary supplementation of Zn largely reversed this enzyme inactivation in various regions of the epididymis. This correlated well with reduced Cd load in calf testes following Zn exposure compared to Cd withdrawal alone. The relationship of the Zn effects on epididymal Cd levels needs investigation. Recently, Saksena et al (1983) demonstrated reduced testicular and epididymal weights, reduced sperm population in both the testis and epididymis as well as reduced serum T levels and loss of fertility in rats given single sc dose of Cd. These changes were partially prevented by single sc dose of zinc given prior to, simultaneously with, or within 2 hr after Cd dosing. However, zinc given 2 hr following Cd administration

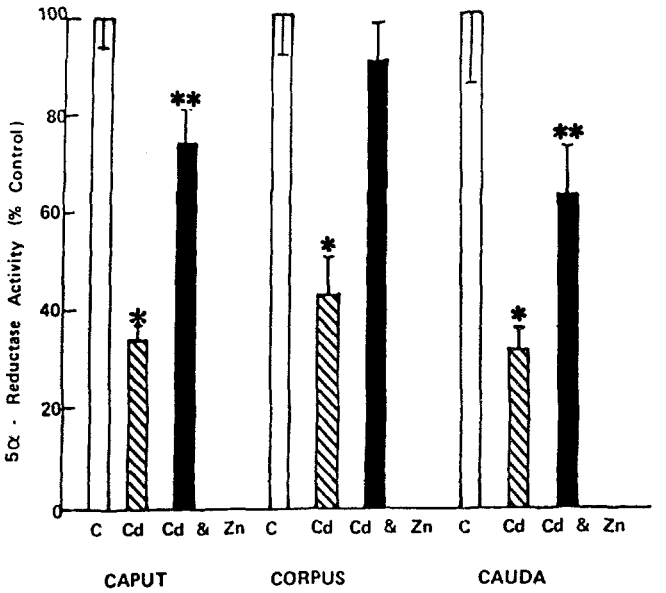

Figure 3. Reversal of cadmium-induced inhibition of epididymal 5 α-reductase in calves by zinc. Values are % of mean control values $\pm$ SD. Open bars represent control (untreated) calves (C), hatched bars represent Cd-treated calves on basal diet (Cd), and dark bars represent Cd-treated calves on zinc diet (Cd & Zn). * indicates significant difference ($P<0.05$) from Cd & Zn group and ** indicates significant difference ($P<0.05$) from C, and Cd groups.

or later failed to reverse Cd-induced testicular and epididymal damage. Pretreatment of rats with zinc by Chen et al (1974), in addition to preventing Cd-induced testicular damage, reduced the

uptake of Cd by the testicular tissue. In our study similar reduction in testicular Cd levels was seen in calves, even when zinc treatment resumed several days following prior Cd loading, indicating the need for longer term treatment with Zn for antidotal purposes.

The results of these studies showed, for the first time, that antidotal treatment of Zn (oral), in mice and calves pre-treated with Cd, is capable of reducing tissue Cd burden in certain target organs without increasing Cd levels in other tissues. Antidotal Zn is also capable of reversing Cd-induced changes in certain clinical chemistry and tissue biochemical parameters that can not be reversed by Cd withdrawal alone. Species difference exist in tissue accumulation of Zn, indicating possible differences in mechanism of reversal of Cd toxicity and accumulation.

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