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PREVENTION BY ZINC OF CADMIUM-INDUCED ALTERATIONS IN PANCREATIC AND HEPATIC FUNCTIONS

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1 Subacute cadmium treatment ($CdCl_2$, 1 mg/kg twice daily for 7 days) in rats disturbs glucose homeostasis as shown by hyperglycemia and decreased glucose tolerance associated with suppression of insulin release, enhancement of hepatic gluconeogenic enzymes and decrease in hepatic glycogen content.

2 Exposure to cadmium increases hepatic cyclic adenosine 3′,5′-monophosphate (cyclic AMP) and this is accompanied by stimulation of basal, adrenaline- as well as glucagon-stimulated form(s) of adenylate cyclase.

3 In contrast to cadmium, subacute administration of zinc ($ZnCl_2$, 2 mg/kg twice daily for 7 days) fails to alter the activities of hepatic gluconeogenic enzymes, cyclic AMP synthesis, as well as glucose clearance and insulin release in response to a glucose load.

4 Zinc, when administered at the same time as cadmium, prevents the cadmium-induced lesions in both hepatic and pancreatic functions.

5 The results are discussed in relation to the possible mechanisms of cadmium toxicity and to the role of sulphydryl groups in the protection exercised by zinc.

Introduction

Cadmium (Cd) is closely related to zinc (Zn) and is found wherever Zn is found in nature. Although Cd and Zn have similar chemical properties, each element affects the mammalian organism diversely and is handled differently by it. Zinc is an essential trace element necessary for the maintenance of normal biochemical functions and whose absorption and turnover are under homeostatic regulatory mechanisms (Halsted, Smith & Irwin, 1974). Cadmium, on the other hand, is a highly toxic, non-essential element that does not obey homeostatic control and accumulates in the organism with age (Schroeder & Balassa, 1961; Cotzias, Borg & Selleck, 1961).

Parizek (1957) demonstrated that Cd-induced testicular degeneration could be prevented by Zn and, more recently, the administration of several other trace metals such as selenium (Se) (Mason & Young, 1967; Merali & Singhal, 1975), iron (Sansi & Pond, 1974), and copper (Hill, Matrone, Payne & Barber, 1963) has also been found to prevent several of the Cd-induced lesions in experimental animals.

It has recently been demonstrated that Cd affects hepatic carbohydrate and cyclic adenosine 3′,5′-monophosphate (cyclic AMP) metabolism (Sporn, Dinu & Stoonescu, 1970; Stowe, Wilson & Goyer, 1972; Singhal, Merali, Kacew & Sutherland, 1974; Merali & Singhal, 1975) as well as pancreatic function in the mouse, rat (Ghafghazi & Mennear, 1973, 1975; Ithakissios, Ghafghazi, Mennear & Kessler, 1975; Merali & Singhal, 1975) and man (Murata, Hirono, Saeki & Nakagawa, 1970). The purpose of the present study was two-fold: (1) to compare the effects of subacute Zn-exposure to those of subacute Cd-intoxication on hepatic carbohydrate and cyclic AMP metabolism as well as pancreatice function and (2) to investigate whether simultaneous administration of Zn can ameliorate the Cd-induced biochemical and functional changes in rats. A 7 day multiple dosing schedule of Cd administration was selected because it was found to induce certain changes in carbohydrate metabolism resembling those produced by chronic Cd-exposure (Singhal *et al.*, 1974). Our results demonstrate that even though Zn, by itself, is without significant effect, it can, when administered at the same time as Cd, effectively protect animals against the Cd-induced alterations in hepatic glucose-production, cyclic AMP metabolism and insulin-secretion.

Methods

Male rats of the Sprague-Dawley strain (200–225 g) employed in this study were maintained on Master Laboratory Chow and water *ad libitum* throughout

the experimental period. One group of rats received cadmium (Cd) ($CdCl_2$, 1 mg/kg, twice daily) while the second group was treated with zinc (Zn) ($ZnCl_2$, 2 mg/kg, twice daily) for 7 days. In the protection experiment, Zn ($ZnCl_2$, 2 mg/kg, twice daily) was administered simultaneously with Cd ($CdCl_2$, 1 mg/kg, twice daily) but at a different site. The daily dose of each compound was administered subcutaneously in two equal aliquots at 12 h intervals, and this schedule was followed for 7 consecutive days. Control rats received equal volumes of 0.9% w/v NaCl solution (saline).

All animals were deprived of food overnight (16 h) and killed 24 h after the administration of the final dose(s). The activities of gluconeogenic enzymes, levels of urea, protein, cyclic AMP, glucose and insulin as well as the glucose tolerance tests were measured as described previously (Merali & Singhal, 1975). The activity of hepatic adenylate cyclase was assayed according to the procedures of Sutherland, Rall & Menon (1962). Adrenaline (50 μM), glucagon (10 μM) and Na fluoride (10 mM) were added to the incubation medium in order to measure the fluoride- and hormone-stimulated form(s) of adenylate cyclase; enzyme activity being expressed as pmol of cyclic AMP formed per mg tissue during a 10 min incubation period.

Statistics

The data were analyzed for significance of differences by Student's *t* test.

Results

Influence of subacute exposure to Cd, Zn or Zn plus Cd on glucose tolerance in intact rats

Blood glucose concentrations were determined immediately before as well as 15, 30 and 60 min after the glucose load (2.0 g/kg, i.p.) (Figure 1). Although Zn failed to alter the resting blood glucose level, exposure to Cd significantly elevated it to 122% of the control value. Simultaneous administration of Zn and Cd resulted in a blood glucose level that was significantly lower than that observed in the group exposed to Cd alone. Peak blood glucose values in response to the glucose load were attained at 15 min in all groups examined; however, this value in Cd-pretreated rats was significantly greater (38%) than that of control animals. Rats pretreated with Zn as well as Cd attained a blood glucose level that was in the same range as that of control animals. Similarly, at 30 and 60 min intervals, Cd-pretreated rats attained a significantly higher blood glucose level than those of Zn- or Zn plus Cd-pretreated rats; the results from the latter two groups not being significantly different from those of the control rats.

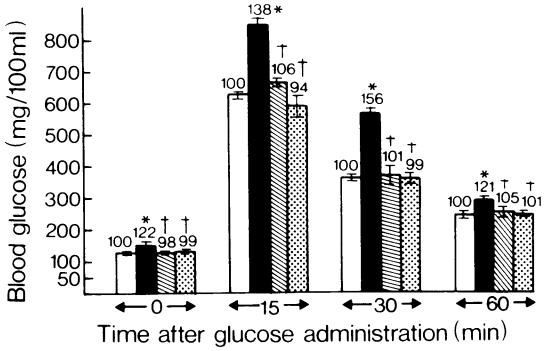

Figure 1 Effects of Zn on Cd-induced loss of glucose tolerance. Glucose tolerance test was carried out 24 h after the administration of the final dose(s) of Cd and/or Zn by giving a glucose load (2.0 g/kg i.p.). Each column represents the mean of 5–6 animals. Vertical lines show s.e. mean. Data are also given above each column as percentages of the values from the respective control groups. Open columns = control; solid columns = Cd, 1 mg/kg twice daily for 7 days; dotted columns = Zn, 2 mg/kg twice daily for 7 days; cross-hatched columns = Zn plus Cd. * Values significantly different from control values at $P < 0.05$. † Values significantly different from the values of Cd-pretreated rats at $P < 0.05$.

Effects of glucose load on serum levels of immuno-reactive insulin of rats exposed to Cd and/or Zn

Figure 2 demonstrates that immediately before glucose administration, serum concentration of immuno-reactive insulin (IRI) of rats pretreated with Zn remained within the normal range. In contrast, the serum IRI level in rats exposed to Cd was considerably depressed. Rats exposed to Cd and Zn simultaneously on the other hand, displayed normal serum IRI content. Glucose administration (2 g/kg) produced prompt elevation in serum IRI concentration, which like the glucose level, peaked at the 15 min time point. In the Zn group, glucose load evoked a normal elevation in serum IRI level; however, in rats pretreated with Cd, statistically significant suppression of glucose-stimulated serum IRI increase was noted at 15, 30 and 60 minutes. In animals receiving Zn plus Cd simultaneously, glucose load elicited a consistently greater increase in serum IRI concentration than it did in the group pretreated with Cd alone, and was not significantly different from the control value.

Table 1 shows the total amount of IRI released in various groups in the 1 h period after glucose load, as calculated by integrating the area under the appropriate curve. It can be seen that the total amount of IRI released by Cd-intoxicated animals was significantly lower than in control animals. Zinc treatment, by itself, did not significantly alter the

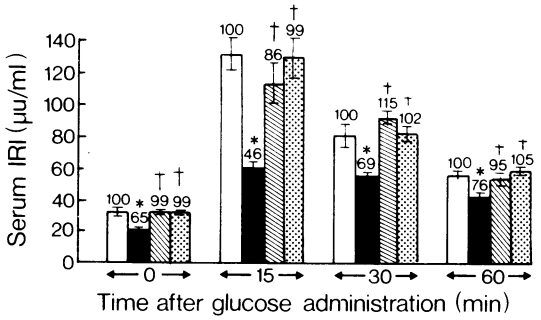

Figure 2 Effect of treatment with Cd, Zn, or Zn plus Cd on immuno-reactive insulin (IRI) release in response to a glucose load. Glucose (2.0 g/kg i.p.) was administered 24 h after the final dose(s) of Cd and/or Zn. Each column represents the mean of 5–6 animals. Vertical lines show s.e. mean. Data are also given above each column as percentages of the values from the respective control groups. Open columns = control; solid columns = Cd, 1 mg/kg twice daily for 7 days; dotted columns = Zn, 2 mg/kg twice daily for 7 days; cross-hatched columns = Zn plus Cd. * Values significantly different from the values of control animals at $P < 0.05$. † Values significantly different from values of Cd-pretreated rats at $P < 0.05$.

amount of IRI released. However, in rats receiving both Zn and Cd simultaneously, significantly more IRI was released than in rats receiving Cd alone.

Activities of hepatic gluconeogenic enzymes after exposure to Cd and/or Zn

Administration of Cd significantly elevated the activities of pyruvate carboxylase (PC) phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-diphosphatase (FD-Pase) and glucose 6-phosphatase (G6-Pase) (Table 2). On the other hand, exposure to Zn failed to produce any significant change in the activities of these four enzymes. When Zn and Cd were administered concurrently, the activities of all four gluconeogenic enzymes were significantly lower than those noted for rats given Cd alone, and were in the same range as control values. In contrast to the gluconeogenic enzymes, Cd-treatment resulted in a reduction of hepatic glycogen level. Although Zn by itself was without any appreciable effect, when given in combination with Cd, it prevented the Cd-induced fall in liver glycogen. Cadmium treatment also increased serum urea by 61% and this rise was effectively prevented by simultaneous administration of Zn, although Zn alone was without any significant effect.

Concentrations of hepatic cyclic AMP in rats exposed to Cd, Zn or both

Subacute exposure to Cd resulted in a marked increase in hepatic cyclic AMP concentration (Table 3). Treatment with Zn alone failed to alter hepatic cyclic AMP significantly. However, when Zn was administered at the same time as Cd, the observed elevation in cyclic AMP level was prevented and the value remained in the range of the control value.

Effects of Cd and Zn on adenylate cyclase activity

Results in Figure 3 demonstrate that Cd-induced elevation of the hepatic cyclic AMP level was accompanied by enhancement in the activity of the cyclic AMP synthesizing enzyme, adenylate cyclase. Adrenaline, fluoride, and glucagon were all able to stimulate adenylate cyclase activity in both normal and Cd-exposed animals; however, in Cd-exposed rats, glucagon and adrenaline could stimulate the enzymatic activity to levels higher than in the corresponding controls whereas fluoride could not. Furthermore, although Zn by itself did not alter the sensitivity of adenylate cyclase to adrenaline,

Table 1 Effects of subacute Cd and/or Zn on integrated immuno-reactive insulin (IRI) levels over a 60 min period after administration of a glucose load

Treatment	*Dose (mg kg^{-1} day^{-1})*	*Estimated total insulin released (min × μu/ml)*	*Percent of control*
Control	0.0	2938 ± 266	(100)
Cd	2 × 1.0	1691 ± 93	(58)*
Zn	2 × 2.0	3010 ± 201	(102)†
Zn + Cd	2 × 2.0 2 × 1.0	2903 ± 220	(99)†

Each value represents the mean ± s.e. mean of 5–6 rats per group.
* Significantly different from control values at $P < 0.05$; † Significantly different from values of Cd-treated group at $P < 0.05$.

glucagon or fluoride, administration of this trace element concurrently with Cd significantly prevented the Cd-stimulated increase in the activity of basal as well as the hormone-stimulated forms of the hepatic enzyme.

Discussion

The results reported in this study demonstrate that the toxic effects of Cd on hepatic metabolism and pancreatic function can be effectively prevented by simultaneous administration of Zn.

In rats, subacute Cd treatment resulted in suppression of pancreatic function as demonstrated by marked reduction of glucose tolerance associated with a decrease in the glucose-stimulated insulin release. The mechanism by which Cd suppresses the insulin secretory response is not clear. Perfusion of isolated pancreas of rat with Cd was found to inhibit the insulin secretory response to glucose, tolbutamide or potassium ions (Ghafghazi & Mennear, 1975)

Table 2 Protective effect of Zn on Cd-induced alterations in hepatic glycogen, gluconeogenic enzymes and serum urea levels

	Treatment			
Parameters examined	*Control*	*Cd*	*Zn*	*Zn+Cd*
PC	306 ± 5.7 (100)	430 ± 13.7 (141)* [100]	316 ± 4.8 (103) [73]†	305 ± 8.8 (100) [71]†
PEPCK	9.8 ± 0.61 (100)	18.1 ± 2.04 (185)* [100]	9.05 ± 0.69 (92) [50]†	10.6 ± 1.09 (108) [58]†
FD-Pase	6.05 ± 0.31 (100)	7.86 ± 0.50 (130)* [100]	6.49 ± 0.17 (107) [83]†	6.24 ± 0.29 (103) [79]†
G6-Pase	2.32 ± 0.13 (100)	2.90 ± 0.11 (125)* [100]	2.35 ± 0.26 (101) [81]†	2.30 ± 0.05 (99) [80]†
Glycogen	2.18 ± 0.2 (100)	1.43 ± 0.09 (65)* [100]	1.95 ± 0.16 (89) [136]†	2.23 ± 0.26 (102) [157]†
Urea	17.6 ± 0.50 (100)	28.4 ± 1.15 (161)* [100]	18.4 ± 0.51 (104) [65]†	18.4 ± 0.51 (104) [65]†

PC=pyruvate carboxylase; PEPCK=phosphoenolpyruvate carboxykinase; FD-Pase=fructose 1,6-di-phosphatase; G6-Pase=glucose 6-phosphatase. Each value represents the mean ± s.e. mean of 5–6 animals. Data are also given in parentheses as percentages of the values of control ( ) or Cd-treated [ ] animals.
* Significantly different from control values at $P < 0.05$; † Significantly different from values of Cd-treated rats at $P < 0.05$.

Table 3 Protective effect of Zn against the Cd-induced increase in hepatic cyclic AMP levels

Treatment	*Dose (mg kg^{-1} day^{-1})*	*Cyclic AMP level (pmol/mg tissue)*	*Percent of control*
Control	0.0	0.74 ± 0.01	(100)
Cd	2 × 1.0	1.36 ± 0.06	(184)*
Zn	2 × 2.0	0.80 ± 0.03	(108)†
Zn + Cd	2 × 2.0 2 × 1.0	0.79 ± 0.02	(106)†

Each value represents the mean ± s.e. mean of 5–6 rats per group.
* Significantly different from control values at $P < 0.05$; † Significantly different from values of Cd-treated group at $P < 0.05$.

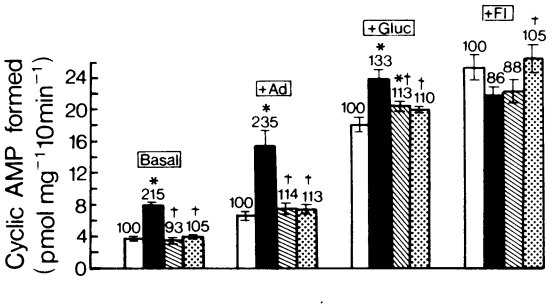

Figure 3 Protection by Zn of Cd-induced alterations in the responsiveness of hepatic adenylate cyclase to adrenaline (Ad), glucagon (Gluc) or fluoride (Fl). Each column represents the mean of 5–6 animals. Vertical lines show s.e. mean. Data are also given above each column as percentages of the values from the respective control groups. Open columns = control; solid columns = Cd; 1 mg/kg twice daily for 7 days; dotted columns = Zn, 2 mg/kg twice daily for 7 days; cross-hatched columns = Zn plus Cd. * Values significantly different from the values of control animals at $P < 0.05$. † Values significantly different from the values of Cd-treated rats at $P < 0.05$.

indicating, that Cd may be inflicting damage directly at the level of pancreatic insulin secretory mechanism(s). Considerable attention has been directed to the possible importance of Zn in the aetiology of diabetes. There appears to be significantly less Zn concentrated in the diabetic pancreas in comparison to the normal tissue (Lowry, Baldwin & Harrington, 1954). Furthermore, several investigators have reported decreased glucose tolerance and glucose-stimulated insulin release in Zn-deficient rats (Huber & Gershoff, 1973; Halstead *et al.*, 1974). This impairment in Zn-deficient animals appears to be similar to that reported in the present study for Cd-intoxicated rats and is of particular interest in view of the present finding that the pancreatotoxic effects of Cd were prevented by simultaneous administration of Zn. This suggests that Cd, as an 'antimetabolite' of Zn (Bremner, 1974), may be suppressing the pancreatic function either by replacing functional Zn or by altering the availability of the essential trace metal; the effect(s) being overcome by the exogenously administered Zn.

There is evidence that SH groups play an important role in the mechanism(s) leading to the release of stored insulin from pancreatic β cells (Havu, 1969; Watkins, Cooperstein & Lazarow, 1970; Watkins & Moore, 1974; Hellman, Idahl, Lernmark, Sehlin & Täljedal, 1975). These observations are of interest since like alloxan (a potent diabetogenic agent), Cd is also a potent SH inhibitor (Havu, 1969). The exact mechanism by which the interaction with pancreatic SH groups results in altered insulin secretory activity is not known. However, studies indicate that both the mono- and dithiol-inhibitors (including Cd) are potent Zn releasers in sculpin islets (Havu, 1969). Since Zn-thiol interactions are known to occur in biological systems, it is possible that exogenous Zn might be affording protection against Cd-induced pancreatic damage by interacting with SH groups (and thus preventing SH-Cd interaction and the consequent loss of Zn). It is of interest that administration of selenium (Se) simultaneously with Cd has also been found to prevent partially the Cd-induced pancreatic damage (Merali & Singhal, 1975). It was suggested that Se might afford protection to the pancreas by preventing the Cd-SH interactions and consequently sparing Cd-induced Zn loss (Merali & Singhal, 1975). This hypothesis may represent a common mechanism by which two diverse elements, Zn and Se, might act to prevent the Cd-induced pancreatic damage.

Insulin reduces the blood glucose level not only by increasing the membranal transport of sugars but also by enhancing the conversion of glucose into glycogen and triglycerides and decreasing the hepatic glucose production (Walaas, Walaas & Grønnerød, 1974). Conversely, insulin deficiency caused either by insulin anti-serum or alloxan treatment increases blood glucose, cyclic AMP as well as glucose synthesis in the liver and reduces its glycogen content (Exton & Park, 1968; Wicks, 1969; Exton, 1972). Since these observations appear to be similar to those noted in Cd-intoxicated animals, it seems likely that the effects of Cd treatment may be due, at least in part, to a lack of insulin. Furthermore, the ability of Zn to prevent various Cd-induced hepatotoxic effects may be related indirectly to its protective effect against Cd-induced insulin diminution.

Cadmium treatment resulted in increased serum urea level. Since Cd is known to cause kidney damage (Stowe *et al.*, 1972), the high blood urea level could be due, at least in part, to renal damage. Simultaneous administration of Zn also prevented Cd-induced uremia.

It has been postulated that Cd is rendered innocuous by its rapid incorporation into a protein called metallothionein whose synthesis is stimulated by Cd (Colucci, Wingé & Krasno, 1975). Recent studies also suggest that Zn may stimulate the formation of a metallothionein-like protein which, in addition to concentrating Zn, may also sequester Cd (Bremner & Davies, 1974). This may therefore represent a detoxifying mechanism.

Cyclic AMP is believed to play an important role in processes of gluconeogenesis as well as glycogenolysis (Sutherland & Robison, 1969; Exton, 1972). In the present study, Cd caused a significant elevation in hepatic cyclic AMP content. Since administration of cyclic AMP has also been found to increase blood glucose and urea levels, enhance synthesis of glucose from non-carbohydrate precursors and lower hepatic glycogen concentration (Menahan & Wieland, 1967;

Exton & Park, 1968; Greengard, 1969; Wicks, 1971), it would appear that the Cd-induced changes in hepatic carbohydrate metabolism resemble those produced by cyclic AMP. Furthermore, since insulin deficiency has been found to elevate the hepatic cyclic AMP concentration (Exton, 1972), the observed increase in cyclic nucleotide levels may be related to the Cd-induced insulin deficiency.

The Cd-induced elevation of hepatic cyclic AMP was accompanied by stimulation of the basal, adrenaline-, as well as glucagon-stimulated form(s) of adenylate cyclase. However, the responsiveness of the enzyme to fluoride remained unaltered. This is concordant with the view that fluoride ion acts through some direct action on the catalytic component and not through the hormone-discriminators (Birnbaumer, Phol & Rodbell, 1971). Intoxication with Cd increased the responsiveness of hepatic adenylate cyclase to adrenaline by a greater degree than to glucagon. This may be relevant to the recent finding that the state of diabetes increases the adrenaline-sensitive adenylate cyclase activity of rat liver (Bitensky, Gorman & Neufeld, 1972). Possibly, the protective effect of Zn against Cd-induced changes in hormone-sensitivity of hepatic adenylate cyclase may be secondary to the prevention of pancreatic damage by Zn.

The present investigation in conjunction with a previous report (Merali & Singhal, 1975) indicates that interactions between Cd, Zn and Se may underlie the observed hepatotoxic and pancreatotoxic effects of Cd in mammals. It is apparent that a clearer understanding of trace element metabolism will be fruitful not only to our understanding of heavy metal toxicities but also to the development of adequate prophylactic and detoxication measures against the inadvertent exposure to heavy metal pollutants.

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