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Changes in carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase activities in the intestine of rats orally exposed to different doses of cadmium

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Abstract

The purpose of the present study was to evaluate the effect of cadmium on some protein digestive and absorption enzymes in rats. Thirty-six rats were grouped into three groups of 12 animals each; one group received deionised water and acted as control. One group received 445 μM Cd and the last group received 890 μM Cd in their drinking water for a period of one month. The results obtained indicate that increasing the level of cadmium from 445 μM to 890 μM in the drinking water of the rats led to 29% and 23% increase in accumulated cadmium in the proximal and distal small intestine respectively. The body weight gain of rats exposed to 445 μM and 890 μM Cd was decreased by about 24% and 43% respectively when compared with the control. The activities of carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase were reduced in the mucosa of the proximal end of the small intestine of cadmium exposed rats. The reduction was dose dependent; with the 890 μM Cd exposed rats displaying the least activities. In the distal small intestine, the activities of these enzymes were restored in the 445 μM Cd exposed rats to levels that were not statistically different ($P > 0.05$) from those observed in the controls. In the 890 μM Cd exposed rats, dipeptidase activity improved by about 80% compared with the activity of the enzyme in the proximal small intestine. Likewise, Na^+/K^+ ATPase activity increased by about 125% compared with the observed level in the proximal small intestine. The study suggests that cadmium given to rats in drinking water compromise protein digestion and absorption of nutrients particularly in the proximal region of small intestine and could account for weight reduction associated with cadmium toxicity.

Introduction

Cadmium has long been known as an industrial and environmental toxicant, causing significant metabolic alterations and injuries of biological systems at different levels (Friberg *et al.* 1974; WHO 1992). Its diverse use in industries has led to its wide distribution in the environment. The interest on this toxicant has been rekindled following the occurrence of the *itai itai* disease in Japan (Horiguchi *et al.* 1996). The oral route is among the most important routes of cadmium exposure in animals and humans. In oral cadmium intoxication the immediate target organ is the gastrointestinal tract, from where the element is readily distributed to other tissues. For this distribution endogenously synthesized intestinal metallothionein (a protein of low molecular weight) has been shown to be important (Elsenhans *et al.* 1997).

Damage to the intestine occurs in oral cadmium toxicity where it has been shown to inhibit intestinal mucosa respiration (Hulinska *et al.* 1988) and also compromise membrane integrity in tissues by generating free radicals thus increasing membrane lipid peroxidation (Bagchi *et al.* 1996; Patra *et al.* 1999; Asagba *et al.* 2002). Some enzymes important for nutrient digestion and absorption in the intestine are membrane bound. The element has been shown to inhibit Na^+/K^+ ATPase, a membrane bound enzyme, in

enterocytes (Mesonero *et al.*1993). The results of the study by Mesonero *et al.* (1993) show that cadmium decreases sucrase activity and D-galactose absorption in the jejunum tissue of rabbits. They attributed the effect to an action mainly located on Na^+ dependent sugar transport of the mucosa border of the intestinal epithelium. In the rat, diets up to 1mmol cadmium induced decrease in the activities of sucrase, lactase, alkaline phosphatase, glycylleucine hydrolase and diamine oxidase in the proximal small intestine (Elsenhans *et al.* 1999).

Reports on the effect of cadmium on intestinal enzymes involved in the digestion and absorption of proteins are lacking. This study reports the effect of different doses of cadmium on carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase activities in different segments of the small intestine of rats.

Materials and methods

Experimental design: Thirty six (ten weeks old) male albino rats of the Wistar strain with an average weight of 100 $\pm$ 3.0 g were housed individually in cages with wire mesh floor to prevent coprophagy. The rats were distributed into three groups with twelve rats per group such that the average weight difference between the groups was less than 0.3 g.The rats in one group were given deionised water and served as control while the rats in the other groups were given 445 and 890 μM Cd respectively in their drinking water as $CdSO_4$. A pilot study in our laboratory had shown that 890 μM Cd was the maximum dose in drinking water tolerated by the rats with quantifiable tissue biochemical changes without fatality. The animals in the control and test groups were treated for one month during which they were fed rat chow and given water freely. The rats were first acclimatized for one week with the diet. All these animal treatment were carried out in accordance with the principles of laboratory animal care of the NIN guide for Laboratory Animal Welfare as contained in the NIN guide for grants and contracts, vol. 14, No. 3, 1985. At the end of the specified treatment period, the animals were fasted for three hours and sacrificed under chloroform anesthesia. The proximal (15 cm from the stomach) and distal (15 cm before the large intestine) portions of the small intestine were immediately recovered and flushed several times with ice-cold normal saline (0.9% NaCl) containing trypsin- and chymotrypsin-inhibitors to recover unabsorbed food. These were centrifuged at 5000 g and the supernatant obtained was used for the assay of carboxypeptidase A.

Mucosa preparation: The portions of the intestine were cut open with a pair of scissors in a glass wash and the mucosa was recovered by carefully scrapping it off the intestinal tissue using a glass slide. It was then homogenized with chilled normal saline in a cold room maintained at 4 °C and centrifuged at 5000 g for ten minutes. The residue was recovered for mucosa cadmium, Na^+/K^+ ATPase and dipeptidase analysis.

Enzyme and protein assays: The activities of carboxypeptidase A and dipeptidase were determined by the method of Appel (1974) and Na^+/K^+ ATPase by the method of Adam-Vizi & Seregi (1982). Carboxypeptidase A was determined with N-carbobenzoxy-glycyl-L-phenylalanine as substrate. The amino acid formed was determined by ninhydrin method (colorimetric method). Dipeptidase was determined using glycylglycine as substrate and the amino acid released was also determined using the ninhydrin colorimetric method. The amount of amino acid was extrapolated from appropriate standards. ATPase activity was measured by the amount of inorganic phosphate liberated following incubation with 25mM disodium ATP. The inorganic phosphate liberated was estimated by the method of Fiske & Subarrow (1925). Total protein was estimated by the method of Lowry *et al.* (1951).

Cadmium analysis

The intestinal cadmium contents were estimated with atomic absorption spectrophotometer (Varian AA 1475) after wet digestion of the tissues. For digestion, 20 ml HNO_3-$HClO_4$ mixture (4:1) was introduced into a beaker containing a weighed portion of the given tissue, followed by heating at 100 °C until the sample was completely digested. Each digest was thereafter diluted to 100 ml with deionised water.

Statistical analysis: The results are expressed as Mean $\pm$ S.E.M. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was used to test for significant differences between the means (Sokal & Rohlf 1969).

Table 1. Cadmium level in proximal and distal small intestine of rats given different doses of cadmium in drinking water.

Group Tissue	Control	445 μM Cd treatment	890 μM Cd treatment	Percentage change between 445 and 890 μM Cd treatment
Proximal small intestine	0.5 ± 0.1^{a}	10.4 ± 0.7^{b}	13.5 ± 0.9^{c}	29.8 %
Distal small intestine	0.6 ± 0.1^{a}	11.8 ± 0.5^{b}	14.6 ± 0.6^{c}	23.7%

Values are means $\pm$ S.E.M. ($n = 12$).
Means of the same row followed by different letters differ significantly ($P < 0.05$).
Cadmium in the tissues is reported as μg cadmium/g wet weight of sample.

Table 2. Water intake, food consumption, weight gain and dry fecal output of rats exposed to different doses of cadmium in drinking water.

Group Parameter	Control	445 μM Cd treatment	890 μM Cd treatment
Food intake (g/rat/day)	13.7 ± 0.3^{a}	13.2 ± 0.4^{a}	12.8 ± 0.5^{a}
Water intake (ml/rat/day)	44.5 ± 6.5^{a}	38.1 ± 5.2^{ab}	30.2 ± 5.0^{b}
Weight gain (g/rat/day)	2.1 ± 0.2^{a}	1.6 ± 0.1^{b}	1.2 ± 0.2^{c}
*Dry fecal output (g/rat/day)	12.2 ± 0.4^{a}	12.7 ± 0.3^{a}	14.2 ± 0.3^{b}

*Values are multiplied by 10.
Values are means $\pm$ S.E.M. ($n = 12$).
Means of the same row followed by different letters differ significantly ($P < 0.05$).

Table 3. Carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase activities in the proximal small intestine of rats exposed to different doses of cadmium in drinking water.

Group Parameter	Control	445 μM Cd treatment	890 μM Cd treatment
Carboxypeptidase A	6.8 ± 0.3^{a}	5.1 ± 0.4^{b}	4.3 ± 0.1^{c}
Dipeptidase	8.6 ± 0.4^{a}	6.3 ± 0.2^{b}	4.4 ± 0.2^{c}
Na^+/K^+ ATPase	12.2 ± 0.5^{a}	8.5 ± 0.4^{b}	4.5 ± 0.2^{c}

Values are given as mean $\pm$ S.E.M. ($n = 12$).
Means of the same row followed by different letters differ significantly ($P < 0.05$).
Carboxypeptidase A and dipeptidase activities are expressed as mmol amino acid released/min/mg protein.
Na^+/K^+ ATPase activity is expressed as mmol inorganic phosphate released/hr/mg protein.

Table 4. Carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase activities in the distal small intestine of rats exposed to different doses of cadmium in drinking water.

Group Parameter	Control	445 μM Cd treatment	890 μM Cd treatment
Carboxypeptidase A	5.5 ± 0.4^{a}	5.2 ± 0.4^{a}	4.0 ± 0.3^{b}
Dipeptidase	8.4 ± 0.3^{a}	8.6 ± 0.4^{a}	7.5 ± 0.3^{b}
Na^+/K^+ ATPase	13.6 ± 0.5^{a}	12.8 ± 0.3^{a}	10.3 ± 0.4^{b}

Values are given as mean $\pm$ S.E.M. ($n = 12$).
Means of the same row followed by different letters differ significantly ($p < 0.05$).
Carboxypeptidase A and dipeptidase activities are expressed as mmol amino acid released/min/mg protein.
Na^+/K^+ ATPase activity is expressed as mmol inorganic phosphate released/hr/mg protein.

Results

The levels of cadmium in the proximal and distal intestine are presented in table1. There was a significant increase in cadmium level in the rats given cadmium in the drinking water in both the proximal and distal small intestine compared with the control given deionised water. The cadmium in both the proximal and distal small intestine was further raised in the 890 μM Cd exposed rats compared with the 445 μM Cd exposed rats. The increase was about 29% in the proximal small intestine and about 23% in the distal small intestine. The study shows that cadmium is accumulated in the small intestine when given in their drinking water and that the distal small intestine relatively accumulates less cadmium than the proximal small intestine in high dose of cadmium.

Water intake, food consumption, weight gain and dry fecal output data are summarized in Table 2. Statistical evaluation did not show significant difference ($P > 0.05$) in the food intake between the groups. Cadmium in the drinking water of rats reduced water intake by the animals and the results show that the reduction was dose dependent. The decrease in water intake however reached a significant level ($P < 0.05$) in the rats given 890 μM Cd. There was a significant decrease ($P < 0.05$) in the weights of rats treated with cadmium compared with the control. There was a 24% and 43% reduction in weight of rats given 445 μM and 890 μM Cd in the drinking water respectively. There was an observed significant increase in the dry fecal output of the animals exposed to 890 μM Cd in drinking water though the animals ate the least.

The activities of carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase in the proximal small intestine of the rats are presented in Table 3. Cadmium significantly reduced the activities of carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase in the proximal end of the small intestine compared with the control. In the rats given 890 μM Cd in their drinking water, the observed decrease was about 100% in the membrane bound enzymes (dipeptidase and Na^+/K^+ ATPase) compared with the control. The result reveals that cadmium reduces enzymes of protein digestion and an important enzyme of absorption.

Table 4 shows the activities of carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase in the distal small intestine of the rats. No statistically significant difference was observed in the activities of these enzymes in the animals given 445 μM Cd compared with the control; however in those given 890 μM Cd the enzyme activities were significantly reduced. Dipeptidase and Na^+/K^+ ATPase activities observed in the distal small intestine were higher than that in proximal small intestine in cadmium treated rats. In dipeptidase activity, about 80% increase was observed while about 125% increase in the activity of Na^+/K^+ ATPase was observed in the rats given 890 μM Cd when the proximal and the distal intestinal values are compared.

Discussion

Cadmium has continued to retain the interest of many researchers following the incidence of itai itai disease in Japan. Contamination of farmland from industrial waste improves the risk of exposure to cadmium. The gastro-intestinal tract is therefore of importance in appreciating its bioavailability in humans. The present study reports the effect of 445 and 890 μM Cd in drinking water on protein digestive enzymes and Na^+/K^+ ATPase important for nutrient absorption in the intestinal mucosa of rats.

In oral cadmium intoxication, cadmium can be rapidly taken up and distributed to the liver and kidney and the degree of uptake is largely dependent on diet type, pH and metallothionein production (Anderson *et al.* 1992). The major site of cadmium uptake in the intestine is not certain, however a low pH of the gastric content emptied into the duodenum is thought to contribute to improved cadmium uptake (Anderson *et al.* 1992). This may account for the observed increase in percentage uptake of cadmium in the proximal small intestine in this study (Table 1) when cadmium given to the rats was increased. The gastro-intestinal tract produces metallothionein which can sequester cadmium and aid its transport to the liver from where it is distributed to other tissues (Elsenhans *et al.* 1997). Earlier studies have shown that the ability of the intestine to produce metallothionein is limited but increase from the proximal to the distal small intestine (Elsenhans *et al.* 1994; Elsenhans *et al.* 1999). This would improve the ability of the distal small intestine to handle cadmium and could have accounted for the relative percentage decreased cadmium in this portion of the small intestine as compared with proximal small intestine when the cadmium given to the rats was increased (Table 1).

The cadmium induced decrease in weight gain of rats observed in this study (Table 2) corroborates the earlier findings of Zikic *et al.* (1998) and Asagba *et al.* (2002). Loss of weight may result from a decrease

in food intake; however similar levels of food were consumed by the rats in the various groups, so the weight loss may be related to nutrient availability. In the 890 μM Cd exposed rats; the about 43% decrease in weight may be connected with severe malabsorption/unavailability of nutrients. The likely increase nutrient loss in the 890 μM Cd treated rats may have accounted for the increased fecal bulk in these animals. Water intake may be related to weight, however the difference in weight between the 890 μM Cd treated rats and the control would not have occurred if it depended only on water.

Cadmium has earlier been shown to be toxic to several tissues including the pancreas (WHO, 1992). Carboxypeptidase A arise from the pancreas, therefore its production may be compromised in cadmium toxicity and this would in part account for the observed low activity of the enzyme in the cadmium treated rats (table 3). Inhibition of some enzymes by cadmium has been attributed to its displacement of zinc which acts as a cofactor in these enzymes (WHO, 1992). Carboxypeptidase A is a zinc peptidase, so the low activity of the enzyme observed in the cadmium treated rats may also be attributed to the zinc displacement ability of cadmium.

Recent studies have shown lipid peroxidation to be an early indicator of cadmium exposure (Bagchi *et al.* 1996; Yin *et al.* 2001). Membrane lipid peroxidation leads to membrane microviscosity and changes in kinetic properties (Halliwell & Gutteridge 1989; Ytrehus & Hegstad 1991) which changes the ultrastructure and integrity of the membrane causing membrane bound enzymes to lose function. Carboxypeptidase A is usually produced in its zymogen form and activated in the small intestine by trypsin which itself is formed from trypsinogen by enteropeptidase found in the brush border of enterocytes. If cadmium affects the ultrastructure of enterocytes, it would also indirectly contribute to the lowered carboxypeptidase A observed in the cadmium treated rats. Dipeptidase is also a membrane bound enzyme, anchored to the membrane of the enterocytes, its activity may also be compromised and may have occasioned its observed low activity in the proximal small intestine of the cadmium treated rats (Table 3). The combined effect of cadmium on carboxypeptidase A and dipeptidase in the proximal end of the small intestine would severely compromise protein digestion and thus its availability. It is therefore not surprising that there was loss in weight and increased fecal output in these rats.

The observed low Na^+/K^+ ATPase in the proximal small intestine in the present study corroborate that of Mesonero *et al.* (1993). Na^+/K^+ ATPase is a membrane bound enzyme; it is highly probable that the observed decrease in the activity of the enzyme in the mucosa of small intestine of cadmium-administered rats was due to the disruption of membrane integrity as a result of the toxic effect of cadmium. Certainly, a study of the histopathology or ultrastructure of the intestinal mucosa would have helped to establish the state of the mucosa of the rats. It also should be acknowledged that other mechanisms have been postulated for cadmium induced inhibition of Na^+/K^+ ATPase activity. One of these mechanisms is based on the ability of cadmium to 'uncouple' oxidative phosphorylation (Yoshioka *et al.* 1995). Since energy is required for the various ATPases, a decrease in ATP concentration would affect the activity of Na^+/K^+ ATPase. Another is the interaction of cadmium with thiol groups of critical proteins and enzymes (Timbrell 1991). Any of these mechanisms could account for the observed decrease in the activity of Na^+/K^+ ATPase in the mucosa of cadmium treated rats (Table 3). Inhibition of Na^+/K^+ ATPase would reduce the uptake of glucose and amino acids by cells (Mesonero *et al.* 1993) and thus contribute to the observed effect of cadmium on the weight gain of the rats.

It is noteworthy that the decrease in the mucosa enzymes of the proximal small intestine was compensated for by the increase in dipeptidase and Na^+/K^+ ATPase activities in the distal small intestine of the rats given cadmium (Table 4). An earlier study (Schneeman & Gallaher, 1980) had reported compensatory rise in enzyme activity in the distal small intestine as an adaptive feature in response to stress. Also metallothionein (a cadmium binding protein) has been shown to play an important role in protecting against cadmium toxicity (Gupta *et al.* 1991; Liu & Klaassen, 1996; Klaassen *et al.* 1999; Liu *et al.* 2000). The ability of the small intestine to produce metallothionein though limited, has been shown to improve longitudinally towards the distal small intestine. The increase in dipeptidase and Na^+/K^+ ATPase activities in the distal small intestine observed in this study (Table 4) may be as a result of an adaptive compensatory rise in these enzymes and enhanced production of metallothionein which will greatly improve protein digestion and absorption. Despite the increase in these distal enzymes, there will be improved chance of loss of nutrients to the large intestine and would contribute to the observed decrease in weight gain and increased

faecal output in the rats exposed to cadmium (Table 2). It should be noted though that the decrease in weight gain and increased faecal output was less severe in rats offered 445 μM Cd relative to those given 890 μM Cd in drinking water. This may not be unconnected with the limited ability of the small intestine to induce the synthesis of metallothionein upon cadmium exposure as the 890 μM Cd treatment could overwhelm the available metallothionein.

In conclusion, our results reveal that exposure of rats to cadmium in drinking water decrease the activities of carboxypeptidase A, dipeptidase and Na^+/K^+ ATPase in the mucosa of the proximal small intestine and that the decrease is dose related. This decrease in the enzymes was however compensated for by improved activities in the distal small intestine in rats exposed to cadmium. Our findings suggest that weight loss in cadmium toxicity is as a result of a compromise in protein digestion and absorption.

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