Physiol. Res. 42: 261–266, 1993

Zinc and Copper in the Tissues and Serum of Cadmium Intoxicated Guinea-Pigs: Influence of Vitamin C

J. KADRABOVÁ, A. MAĎARIČ, E. GINTER

Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

Received January 21, 1993
Accepted February 24, 1993

Summary

Cadmium in the dose of 1 mg/animal/day was administered to guinea-pigs in the form of $CdCl_2$ dissolved in drinking water during short-term (5 weeks) and subchronic (12 weeks) experiments. Both the control and cadmium-treated groups were divided into two subgroups, according to low (2 mg/animal/day) and high (100 mg/animal/day) vitamin C intake. Subchronic cadmium treatment caused copper deficiency indicated by a dramatic decrease of copper concentration in the liver and serum and by its moderate decrease in the testes and brain. Cadmium significantly increased zinc concentrations in the kidneys during the whole experiment and decreased the level of zinc in the serum after subchronic cadmium treatment. In the control groups, the levels of zinc and copper in the serum were lowered after 5 weeks of high vitamin C doses. High doses of vitamin C in cadmium-treated guinea-pigs decreased the levels of copper in the testes, brain and serum. These observations suggest that cadmium intake in relatively high doses might potentiate the development of copper deficiency, and high doses of vitamin C aggravate, to a certain extent, copper depletion in some important organs and serum of guinea-pigs.

Key words

Cadmium – Zinc – Copper – Copper deficiency – Vitamin C – Guinea-pigs

Introduction

Essential trace elements, zinc and copper, are components of many important enzymes. Both elements are involved in carbohydrate or lipid metabolism and in immune functions. On the other hand, cadmium is a toxic heavy metal. Cadmium accumulates in various tissues and its concentrations in the kidneys and liver are extremely high compared with other organs (Kostial 1986, Kadrabová *et al.* 1992). The fate and distribution of toxic cadmium and essential zinc and copper are closely related to metallothionein (Bremner and Beattie 1990). All three metals are capable of inducing metallothionein synthesis. The subsequent binding of cadmium ions to metallothionein is a major detoxifying mechanism for cadmium in the gut. Metallothionein is involved in the homeostatic control of zinc and copper absorption and metabolism. Disturbances in zinc and copper metabolism in the liver and kidneys have been found after cadmium administration to experimental animals (Chmielnicka *et al.* 1985, 1989). Chronic cadmium intoxication resembled zinc and copper deficiencies resulting from antagonistic interactions between toxic cadmium and essential zinc and copper. Supplementation of the diet with zinc or vitamin C has a protective effect against the accumulation and toxic effects of cadmium (Jacobs *et al.* 1983, Chmielnicka and Cherian 1986). Vitamin C as a chelating and reducing agent interacts with metallic ions and may therefore promote or inhibit their absorption, retention and excretion. High doses of vitamin C antagonize the metabolism of copper in various animal species (Milne and Omaye 1980, Johnson 1986). The data from human studies are not consistent (Milne *et al.* 1988, Jacob *et al.* 1987)

This study was undertaken to examine the effects of orally administered cadmium and different doses of vitamin C on the metabolism of zinc and copper in guinea pigs.

Methods

Tricoloured male guinea-pigs (Velaz, Prague), with initial body weight ranging from 350-450 g, were placed in plastic cages with wood chip bedding under standard laboratory conditions. Animals were acclimated for at least 14 days prior to the experiment. During the adaption period, guinea-pigs were fed a standard laboratory diet (Mok Velaz, Prague) with the addition of vegetables. Thereafter, the animals were fed a diet free of vitamin C : oat flakes - 490 g/kg, milk powder - 300 g/kg, butter - 100 g/kg, sugar - 100 g/kg and NaCl - 10 g/kg. According to the cadmium intake in the form of $CdCl_2$, (Merck) in the drinking water, guinea-pigs were divided into two main groups: control and cadmium-treated. Control groups received low: 2 mg/animal/24 h (-C,-Cd) and high: 100 mg/animal/24 h (+C,-Cd) dose of vitamin C in drinking water. Animals in the cadmium-treated groups were given 1 mg cadmium /animal/24 h and at the same time low (-C,+Cd) and high doses of vitamin C (+C,+Cd). The diet contained 26.3 mg/kg of zinc, 1.7 mg/kg of copper and 0.041 mg/kg of cadmium as analyzed by atomic absorption spectrometry. The animals had access to tap water and the diet *ad libitum*. The experiment was divided into a short-term (5 weeks) and subchronic (12 weeks) cadmium treatment. After overnight fasting, the animals were killed by decapitation and blood was collected from the neck wound into a tube via a plastic funnel. The liver, kidneys, testes and also the heart and brain after 12 weeks were excised and rinsed in ice-cold saline solution. Tissue samples weighing approximately 1 g were stored at −20 °C. The serum was separated and frozen after centrifugation. Dry mineralisation of the tissues was performed using the Apion device (Tessek). The mineralisation consisted in leading the superoxidative gas mixture (oxygen, nitrogen oxides and ozone) into containers with the samples heated to 400 °C. The whole mineralisation procedure lasted 20 h. After mineralisation, white ash was dissolved in 2 M HNO_3 and deionized water was added up to 25 ml. Zinc and copper in the organs were determined by flame atomic absorption spectrophotometer (model PU 9400X, Unicam Analytical Systems). The standard procedure (Perkin-Elmer 1976) and the STAT flame atomic absorption spectrophotometry technique (Brown and Taylor 1985) were used. Zinc and copper in the serum were measured directly by flame atomic absorption spectrophotometry after simple dilution with deionized water (Salmela and Vuori 1984). Bovine liver 12-2-01, obtained from the Institute of Metrology (Bratislava) was digested and analyzed along with samples to verify accuracy. The mean values obtained for 6 determination were $25.9 \pm 0.8\ \mu g/g$ for copper and $162.3 \pm 1.6\ \mu g/g$ for zinc while the certified values are $26.3 \pm 1.6\ \mu g/g$ and $162.0 \pm 6.0\ \mu g/g$ respectively.

All data were subjected to analysis of variance and regression analysis. Differences with $P<0.05$ were considered as statistically significant.

Results

There was no significant difference in body weight among particular groups in the 5th week of the experiment. After subchronic cadmium treatment, the body weight of animals in cadmium-treated groups was significantly lower. Relative liver weights were significantly increased from 3.20 ± 0.15 g/100 g b.w. (-C,-Cd) to 5.04 ± 0.39 g/100 g b.w. (-C,+Cd) by the subchronic cadmium treatment. Vitamin C had no significant influence on relative liver weight in either control or cadmium-treated groups. Both short-term and subchronic cadmium treatments significantly increased zinc content in the kidneys (Table 1). A positive correlation between zinc and cadmium concentrations in the kidneys was found (5 weeks: $r = 0.733$, $P<0.001$; 12 weeks: $r = 0.798$, $P<0.0001$). In the liver, a more pronounced correlation was found only at the end of the experiment at 12 weeks: $r = 0.628$, $P<0.001$). Cadmium slightly decreased serum zinc in the 5th week, but a significant decrease by about 33 % was observed after subchronic cadmium treatment.

In the control groups, high doses of vitamin C significantly decreased the level of zinc in the serum by about 24 % in week 5. The zinc contents in the liver, kidneys, testes, brain and heart were unchanged by vitamin C at all times. In the cadmium-treated groups, vitamin C significantly increased zinc concentrations in the liver after the short-term treatment. Neither cadmium nor vitamin C influenced the zinc content in the testes and brain. Cadmium treatment caused a significant decrease of copper concentrations in the liver and serum by about 36 % and 65 % after the short-term treatment and by about 78 % and 70 % respectively after subchronic cadmium treatment (Table 2).

A strong negative correlation between cadmium and copper concentrations in the liver was observed particularly at the end of cadmium treatment (5 weeks: $r = -0.550$, $P<0.001$; 12 weeks: $r = -0.874$, $P<.00001$). In the 5th week of cadmium treatment, the copper content was significantly decreased by about 40 % in the testes and a negative correlation was found between cadmium and copper concentrations (5 weeks: $r = -0.608$, $P<0.001$; 12 weeks: $r = -0.627$, $P<0.001$). In subchronic cadmium treatment, the copper concentration also dropped in the brain. These results indicate that cadmium treatment caused copper depletion in guinea-pigs.

In the control groups, vitamin C significantly decreased the serum copper levels at week 5 (Table 2). During the whole study, copper concentrations in all the examined organs were not altered by vitamin C in the control groups, except the liver. At week 5, vitamin

C significantly increased the copper content in liver tissue in the cadmium-treated group.

At the end of the experiment, a high dose of vitamin C decreased the copper levels in the testes, brain and serum by about 51 %, 26 % and 45 % respectively in the cadmium-treated groups (Table 2). The heart copper concentration was unchanged by both cadmium and vitamin C treatment.

Table 1
Zinc levels in the organs and serum of guinea-pigs after short-term and subchronic cadmium treatment

Weeks of Expt.	Groups	Serum μg/ml	Kidneys mg/kg w.w.	Liver mg/kg w.w.	Testes mg/kg w.w.	Heart mg/kg w.w	Brain mg/kg w.w.
5	−C, −Cd	1.58 ± 0.04^{b}	20.26 ± 0.84^{a}	25.75 ± 0.96^{a}	14.20 ± 0.40^{a}	not analysed	not analysed
	+C, −Cd	1.21 ± 0.06^{a}	20.75 ± 1.41^{a}	26.08 ± 0.88^{a}	13.56 ± 0.37^{a}		
	−C, +Cd	1.30 ± 0.11^{ab}	28.33 ± 1.02^{b}	$27.02 \pm 1.3l^{a}$	12.66 ± 0.55^{a}		
	+C, +Cd	1.41 ± 0.11^{ab}	27.26 ± 0.67^{b}	33.81 ± 1.53^{b}	12.77 ± 0.32^{a}		
12	−C, −Cd	1.66 ± 0.08^{bc}	22.41 ± 1.35^{a}	23.79 ± 0.89^{a}	13.66 ± 0.2^{5a}	16.34 ± 0.83^{a}	12.10 ± 0.45^{a}
	+C, −Cd	1.77 ± 0.06^{c}	23.20 ± 1.16^{a}	24.98 ± 0.88^{a}	14.08 ± 0.36^{a}	16.61 ± 0.74^{a}	12.43 ± 0.13^{a}
	−C, +Cd	1.08 ± 0.05^{a}	35.28 ± 3.21^{b}	28.49 ± 1.61^{ab}	13.96 ± 0.18^{a}	20.14 ± 0.29^{b}	12.73 ± 0.36^{a}
	+C, +Cd	1.38 ± 0.09^{ab}	32.61 ± 0.66^{b}	30.10 ± 1.09^{b}	14.15 ± 0.45^{a}	18.52 ± 0.48^{ab}	11.45 ± 0.39^{a}

Each value represents the mean ± S.D. for 8 animals per group.
-C,-Cd: low vitamin C,+C,-Cd: high vitamin C,-C,+Cd: low vitamin C, Cd-treated,+C,+Cd: high vitamin C, Cd-treated,^{a,b,c} - values in the same column not sharing a common superscript are significantly different ($P<0.05$) from values for week 5 or week 12.

Table 2
Copper concentrations in organs and serum of guinea-pigs after short-term and subchronic cadmium treatment

Weeks of Expt.	Groups	Serum μg/ml	Kidneys mg/kg w.w.	Liver mg/kg w.w.	Testes mg/kg w.w.	Heart mg/kg w.w.	Brain mg/kg w.w.
5	−C, −Cd	0.72 ± 0.03^{c}	3.53 ± 0.14^{a}	3.95 ± 0.24^{b}	1.74 ± 0.15^{b}	not analysed	not analysed
	+C, −Cd	0.59 ± 0.04^{b}	2.58 ± 0.17^{a}	4.29 ± 0.13^{b}	1.49 ± 0.08^{ab}		
	−C, +Cd	0.25 ± 0.01^{a}	2.92 ± 0.31^{a}	2.51 ± 0.15^{a}	1.04 ± 0.08^{a}		
	+C, +Cd	0.25 ± 0.02^{a}	3.43 ± 0.30^{a}	3.81 ± 0.16^{b}	1.33 ± 0.13^{ab}		
12	−C, −Cd	0.66 ± 0.06^{c}	2.95 ± 0.17^{b}	4.18 ± 0.38^{b}	0.71 ± 0.03^{b}	2.86 ± 0.10^{a}	2.25 ± 0.13^{c}
	+C, −Cd	0.60 ± 0.04^{c}	2.55 ± 0.12^{ab}	3.96 ± 0.26^{b}	0.70 ± 0.05^{b}	3.26 ± 0.29^{a}	2.07 ± 0.07^{bc}
	−C, +Cd	0.20 ± 0.02^{b}	2.40 ± 0.24^{ab}	0.92 ± 0.11^{a}	0.59 ± 0.05^{b}	3.43 ± 0.11^{a}	1.88 ± 0.09b
	+C, +Cd	0.11 ± 0.03^{a}	2.25 ± 0.11^{a}	0.95 ± 0.17^{a}	0.29 ± 0.02^{a}	3.49 ± 0.11^{a}	1.39 ± 0.07^{a}

Footnotes are the same as in Table 1

Discussion

The concentrations of zinc and copper in different organs are generally constant. The change in their concentrations in tissues occurs when the cadmium content slightly exceeds the physiological concentrations of zinc and copper (Chmielnicka *et al.* 1985, 1989). In this study, cadmium treatment increased the zinc concentrations in the liver and kidneys. Such an effect has also been found by other investigators (Waalkes 1986, Friel *et al.* 1987). In the liver metallothionein, induced by cadmium, 2-3 bound zinc ions could be detected among the total of 7 metal ions bound (Otvos and Armitage 1980). A more pronounced increase of zinc concentration in the kidneys and a decrease in serum zinc may be explained by the involvement of metallothionein in the transport of cadmium in the plasma from the liver to the kidneys (Garvey and Chang 1981). The decrease in serum zinc by 50 % and the increase in the liver zinc concentration after long-term cadmium administration was observed by Hopf *et al.* (1990). The response of the zinc concentration in the testes was somewhat different. The zinc levels were not affected by the exposure to cadmium. Unlike the liver and kidneys, which are rich in metallothionein, the testes appears to be deficient in metallothionein which cannot be induced in this tissue (Waalkes and Perantoni 1986).

In the present work, subchronic oral cadmium treatment induced signs of copper deficiency in guinea-pigs. Dietary cadmium competitively inhibits the intestinal absorption and utilization of copper. The cadmium-copper antagonism is mediated *via* intestinal metallothionein. Perorally administered cadmium induces this metal binding protein in mucosal cells and metallothionein accumulates when cadmium is in excess. The cadmium-induced metallothionein binds copper much more strongly that zinc or cadmium, so that even low concentrations of copper compete favourably for the metal binding sites. The resulting copper-metallothionein is poorly absorbed, creating copper deficiency (Bremner and Beattie 1990). It is well established that the liver plays a major role in copper homeostasis. Hepatic copper concentration is commonly used to assess the copper status. Cadmium treatment has been reported to have an antagonistic effect on copper stores in the liver (Bremner 1979). We observed a dramatic decrease in the liver copper concentration after 12 weeks of cadmium treatment. Copper deficiency in our animals was also confirmed by the very low copper level in the serum and by a moderate decrease of copper concentrations in the brain and testes.

Kidneys are regarded as a critical organ of cadmium exposure (Kjellström 1986). Impaired kidney function and kidney lesions have been reported in studies of copper deficiency (Saari *et al.* 1990, Fell *et al.* 1987). When cadmium induces copper deficiency, its harmful effect on the kidneys may be enhanced. This effect of cadmium on tissues copper levels is more pronounced depending on the duration of cadmium exposure.

The effect of a different intake of vitamin C on zinc concentrations in most tissues was not pronounced, with the exception of the drop in the serum zinc in the control groups and elevated zinc content in the liver of cadmium-treated animals given high doses of vitamin C.

Tsao *et al.* (1990) described a decrease in serum zinc concentrations 4 weeks after intraperitoneal injections of vitamin C in guinea-pigs. Ascorbate administration has been postulated to increase zinc incorporation from the plasma into the liver, possibly by enhancing zinc transport into hepatic cells (Onosaka *et al.* 1987).

Dietary supplementation of vitamin C impairs the uptake and utilization of copper (Johnson and Murphy 1988). Even when dietary copper intake is adequate, vitamin C supplementation may antagonize its utilization. In experimental animals, vitamin C administration decreased copper levels in the serum (Milne and Omaye 1980, Smith and Bidlack 1980) which has been confirmed in our study. On the other hand, vitamin C significantly increased hepatic copper levels in the cadmium-treated group in the short-term experiment. The reason is still not clear. As vitamin C induces the synthesis of metallothionein in the liver (Onosaka *et al.* 1987), we suppose that, during the short-term cadmium treatment, metallothionein induction by cadmium is not saturated, and high doses of vitamin C enhance further metallothionein synthesis so that more copper may be bound. At the end of the experiment, vitamin C further depleted copper concentrations in the testes, brain and serum in the cadmium-treated groups. Thus vitamin C, to a certain extent, may aggravate copper deficiency. The impairment of the copper status by vitamin C may be caused by a decrease of copper absorption or by an increase of copper turnover (Johnson 1986). To what extent the exposure to cadmium impairs zinc and copper metabolism depends on the zinc and copper status of the body and on the intake of both micronutrients from the diet which, in turn, may influence the accumulation and toxic effects of cadmium. When an organism is deficient of zinc and copper, the retention of cadmium and its toxic effects are more pronounced and might lead to further aggravation of zinc and particularly copper deficiencies.

References

BREMNER I., BEATTIE J.H.: Metallothionein and the trace minerals. *Annu. Rev. Nutr.* **10**: 63–83, 1990.

BREMNER I.: Toxicity of cadmium, zinc and molybdenum, and their effect on copper metabolism. *Proc. Nutr. Soc.* **38**: 235–242, 1979.

BROWN A.A., TAYLOR A.: Application of slotted quartz tube flame atomic-absorption spectrometry to the analysis of biological samples. *Analyst* **110**: 579–584, 1985.

CHMIELNICKA J., CHERIAN M.G.: Environmental exposure to cadmium and factors affecting trace-element metabolism and metal toxicity. *Biol. Trace Element Res.* **10**: 163–175, 1986.

CHMIELNICKA J., BEM E.M., BRZEZNICKA E.A., KASPAREK M.: The tissue disposition of zinc and copper following repeated administration of cadmium and selenium to rats. *Environ. Res.* **37**: 419–424, 1985.

CHMIELNICKA J., HALATEK T., JEDLINSKA U.: Correlation of cadmium-induced nephropathy and the metabolism of endogenous copper and zinc in rats. *Ecotoxicol.Environ. Safety* **18**: 268–276, 1989.

FELL B.F., FARQUHARSON C., RIDDOCH G.: Kidney lesions in copper-deficient rats. *J. Comp. Pathol.* **97**: 187–196, 1987.

FRIEL J.K., BORGMAN R.F., CHANDRA R.K.: Effect of chronic cadmium administration on liver and kidney concentrations of zinc, copper, iron, manganese and chromium. *Bull. Environ. Contam. Toxicol.* **38**: 588–593, 1987.

GARVEY J.S., CHANG C.C.: Detection of circulating metallothionein in rats injected with zinc or cadmium. *Science* **214**: 805–810, 1981.

HOPF G., BÖCKER R., BISCHOFF J., WERMER M.G., ESTLER C.J. : Investigation into the combined effects of ethanol and cadmium on rat liver and kidneys. *Arch. Toxicol.* **64**: 470–473, 1990.

JACOB R.A., SKALA J.H., OMAYE S.T., TURNLUND J.R.: Effect of varying ascorbic acid intakes on copper absorption and ceruloplasmin levels of young men. *J. Nutr.* **117**: 2109–2115, 1987.

JACOBS R.M., JONES A.O.L., FOX M.R.S., LENER J.: Effect of dietary zinc, manganese and copper on tissue accumulation of cadmium by Japanese quail. *Proc. Soc. Exp. Biol. Med.* **172**: 34–38, 1983.

JOHNSON M.A., MURPHY C.L.: Adverse effects of high dietary iron and ascorbic acid copper status in copper-deficient and copper-adequate rats. *Am. J. Clin. Nutr.* **47**: 96–101, 1988.

JOHNSON M.A.: Interaction of dietary carbohydrate, ascorbic acid copper with the development of copper deficiency in rats. *J. Nutr.* **116**: 802–815, 1986.

KADRABOVÁ J., MAĎARIČ A., GINTER E.: The effect of ascorbic acid on cadmium accumulation in guinea pig tissues. *Experientia* **48**: 989–991, 1992.

KJELLSTRÖM T.: Critical organs, critical concentrations, and whole body dose-response relationships. In : *Cadmium and Health,* Vol.II, L. FRIBERG, C.G. ELINDER, T. KJELLSTRÖM, G.F. NORDBERG, (eds), CRC Press, Boca Raton, Florida, 1986, pp. 231–246.

KOSTIAL K.: Cadmium. In : *Trace Elements in Human and Animal Nutrition,* Vol.2, W. MERTZ, (ed.) Academic Press, Orlando, 1986, pp. 319–345.

MILNE D.B., KLEVAY L.M., HUNT J.R.: Effects of ascorbic acid supplements and a diet marginal in copper on indices of copper nutrition in women. *Nutr. Res.* **8**: 865–873, 1988.

MILNE D.B., OMAYE S.T.: Effect of vitamin C on copper and iron metabolism in the guinea pig. *Int. J. Vit. Nutr. Res.* **50**: 301–308, 1980.

ONOSAKA S., KAWAKAMI D., MIN K.S., OO-ISCHI K., TANAKA K.: Induced synthesis of metallothionein by ascorbic acid in mouse liver. *Toxicology,* **43**: 251–259, 1987.

OTVOS J.D., ARMITAGE I.M.: Structure of the metal clusters in rabbit liver metallothionein. *Proc. Natl. Acad. Sci. USA* **77**: 7094–7098, 1980.

PERKIN-ELMER CORPORATION. *Analytical Methods for Atomic Absorption Spectrophotometry.* Norwolk, CT, 1976.

SAARI J.T., REEVES P.G., NOORDEWIER B., HALL C.B., LUKASKI H.C.: Cardiovascular but not renal effects of copper deficiency are inhibited by dimethyl sulfoxide. *Nutr. Res.* **10**: 467–477, 1990.

SALMELA S., VUORI E.: Improved direct determination of copper and zinc in a single serum dilution by atomic absorption spectrophotometry. *Atomic Spectr.* **5**: 146–149, 1984.

SMITH C.H., BIDLACK W.R.: Interrelationships of dietary ascorbic acid and iron on the tissue distribution of ascorbic acid, iron, and copper in female guinea pigs. *J. Nutr.* **110**: 1398–1408, 1980.

TSAO C.S., LEUNG P.Y, YOUNG M.: Levels of minerals in serum and urine of guinea pigs following intraperitoneal administration of ascorbate. *Int. J. Vit. Nutr. Res.* **60**: 121–125, 1990.

WAALKES M.P.: Effect of dietary zinc deficiency on the accumulation of cadmium and metallothionein in selected tissues of the rat. *J. Toxicol. Environ. Health* **18**: 301–313, 1986.

WAALKES M.P., PERANTONI A.: Isolation of a novel metal-binding protein from rat testis. Characterization and distinction from metallothionein. *J. Biol. Chem.* **261**: 13079 – 13103, 1986.

Reprint Requests
Dr. J. Kadrabová, Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic.