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

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Summary
Cadmium in the dose of 1 mg/animal/day was administered to guinea-pigs in the form of CdCl₂ 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 CdCl₂ (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 HNO₃ 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±0.8 µg/g for copper and 162.3±1.6 µg/g for zinc while the certified values are 26.3±1.6 µg/g and 162.0±6.0 µg/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<0.00001). 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

<table>
<thead>
<tr>
<th>Weeks of Groups</th>
<th>Serum μg/ml</th>
<th>Kidneys mg/kg w.w.</th>
<th>Liver mg/kg w.w.</th>
<th>Testes mg/kg w.w.</th>
<th>Heart mg/kg w.w.</th>
<th>Brain mg/kg w.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expt.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-C, -Cd</td>
<td>1.58 ± 0.04^b</td>
<td>20.26 ± 0.84^a</td>
<td>25.75 ± 0.96^a</td>
<td>14.20 ± 0.40^a</td>
<td>not analysed</td>
<td>not analysed</td>
</tr>
<tr>
<td>5 +C, -Cd</td>
<td>1.21 ± 0.06^a</td>
<td>20.75 ± 1.41^a</td>
<td>26.08 ± 0.88^a</td>
<td>13.56 ± 0.37^a</td>
<td>12.66 ± 0.55^a</td>
<td>12.01 ± 0.32^a</td>
</tr>
<tr>
<td>-C, + Cd</td>
<td>1.30 ± 0.11^ab</td>
<td>28.33 ± 1.02^b</td>
<td>27.02 ± 1.13^a</td>
<td>12.36 ± 0.55^a</td>
<td>12.66 ± 0.55^a</td>
<td>12.01 ± 0.32^a</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>1.41 ± 0.11^ab</td>
<td>27.26 ± 0.67^b</td>
<td>33.81 ± 1.53^b</td>
<td>12.77 ± 0.32^a</td>
<td>12.66 ± 0.55^a</td>
<td>12.01 ± 0.32^a</td>
</tr>
<tr>
<td>12 +C, -Cd</td>
<td>1.66 ± 0.08^bc</td>
<td>22.41 ± 1.35^a</td>
<td>23.79 ± 0.89^a</td>
<td>13.66 ± 0.25^a</td>
<td>16.34 ± 0.83^a</td>
<td>12.10 ± 0.45^a</td>
</tr>
<tr>
<td>-C, + Cd</td>
<td>1.77 ± 0.06^c</td>
<td>23.20 ± 1.16^a</td>
<td>24.98 ± 0.88^a</td>
<td>14.08 ± 0.36^a</td>
<td>16.61 ± 0.74^a</td>
<td>12.43 ± 0.13^a</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>1.08 ± 0.05^a</td>
<td>35.28 ± 3.21^b</td>
<td>28.49 ± 1.61^ab</td>
<td>13.96 ± 0.18^a</td>
<td>20.14 ± 0.29^b</td>
<td>12.73 ± 0.36^a</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>1.38 ± 0.09^ab</td>
<td>32.61 ± 0.66^b</td>
<td>30.10 ± 1.09^b</td>
<td>14.15 ± 0.45^a</td>
<td>18.52 ± 0.48^ab</td>
<td>11.45 ± 0.39^a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Weeks of Groups</th>
<th>Serum μg/ml</th>
<th>Kidneys mg/kg w.w.</th>
<th>Liver mg/kg w.w.</th>
<th>Testes mg/kg w.w.</th>
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<td><strong>Expt.</strong></td>
<td></td>
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</tr>
<tr>
<td>-C, -Cd</td>
<td>0.72 ± 0.03^c</td>
<td>3.53 ± 0.14^a</td>
<td>3.95 ± 0.24^b</td>
<td>1.74 ± 0.15^b</td>
<td>not analysed</td>
<td>not analysed</td>
</tr>
<tr>
<td>5 +C, -Cd</td>
<td>0.59 ± 0.04^b</td>
<td>2.58 ± 0.17^a</td>
<td>4.29 ± 0.13^ab</td>
<td>1.49 ± 0.08^ab</td>
<td>12.01 ± 0.32^a</td>
<td>12.66 ± 0.55^a</td>
</tr>
<tr>
<td>-C, + Cd</td>
<td>0.25 ± 0.01^a</td>
<td>2.92 ± 0.31^a</td>
<td>2.51 ± 0.15^a</td>
<td>1.04 ± 0.08^a</td>
<td>12.01 ± 0.32^a</td>
<td>12.66 ± 0.55^a</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>0.25 ± 0.02^a</td>
<td>3.43 ± 0.30^a</td>
<td>3.81 ± 0.16^b</td>
<td>1.33 ± 0.13^ab</td>
<td>12.01 ± 0.32^a</td>
<td>12.66 ± 0.55^a</td>
</tr>
<tr>
<td>12 +C, -Cd</td>
<td>0.66 ± 0.06^c</td>
<td>2.95 ± 0.17^b</td>
<td>4.18 ± 0.38^b</td>
<td>0.71 ± 0.03^a</td>
<td>2.86 ± 0.10^a</td>
<td>2.25 ± 0.13^c</td>
</tr>
<tr>
<td>-C, + Cd</td>
<td>0.60 ± 0.04^c</td>
<td>2.55 ± 0.12^ab</td>
<td>3.96 ± 0.26^b</td>
<td>0.70 ± 0.05^ab</td>
<td>3.26 ± 0.29^b</td>
<td>2.07 ± 0.07^bc</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>0.20 ± 0.02^b</td>
<td>2.40 ± 0.24^ab</td>
<td>0.92 ± 0.11^a</td>
<td>0.59 ± 0.05^b</td>
<td>3.43 ± 0.11^a</td>
<td>1.88 ± 0.09b</td>
</tr>
<tr>
<td>+C, + Cd</td>
<td>0.11 ± 0.03^a</td>
<td>2.25 ± 0.11^a</td>
<td>0.95 ± 0.17^a</td>
<td>0.29 ± 0.02^a</td>
<td>3.49 ± 0.11^a</td>
<td>1.39 ± 0.07^a</td>
</tr>
</tbody>
</table>

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 than 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


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