# The effect of ascorbic acid on cadmium accumulation in guinea pig tissues

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*Abstract.* Accumulation of cadmium in organs of guinea pigs after subchronic oral cadmium treatment (1 mg Cd/animal/24 h) was in the following order: kidneys > liver > heart > testes > brain. The preventive effects of high doses of ascorbic acid (AA) against cadmium deposition were more pronounced in the testes, heart and brain, and in the kidney only after short-term cadmium treatment. Ascorbic acid had no protective effect on cadmium accumulation in the liver.
*Key words.* Cadmium; ascorbic acid; guinea pig.

Cadmium is a xenobiotic metal whose metabolism is characterized by poor homeostatic control and excessive retention in the body[1]. The toxic influence of Cd on various organs (liver, kidneys, lungs) has been confirmed both in experimental animals and in humans[2–4]. Cadmium enters the soil with phosphatic fertilizers, sewage sludge and air pollutants. In comparison with other heavy metals it has a great mobility in the soil and is taken up by plants in varying degrees[5]. Its intake by humans comes about mainly via the food chain[6]. Oral absorption of environmental Cd is poor (about 5%), but long-term cadmium exposure leads to its accumulation in critical organs (kidneys, liver)[7] and the metal has a long biological half-life of 10–30 years[8].

Nephrotoxicity is regarded as the 'critical' effect in human exposure to Cd[9]. In addition, long-term environmental exposure to cadmium may lead to hypertension[10–12] and Cd may, by elevating blood pressure or through other mechanisms, contribute to the pathogenesis of cardiovascular diseases, which are the leading cause of death in most industrialized countries[13,14]. Since the changes in the organism caused by Cd are irreversible the question of primary prevention is of great importance. Efficient prevention may be achieved by increasing the content of nutrients (vitamins, essential trace elements) in the diet. In general, suboptimal intakes of the essential nutrients exacerbate the adverse effects of toxic elements[15]. Although data on certain protective effects of ascorbic acid (AA) against the accumulation of Cd in the organs are available, they are based mainly on the results of experiments with rats[16,17].

The guinea pig was chosen as an experimental model which allows extrapolation of the experimental results to man. Guinea pigs, like man, do not synthesize endogenous ascorbic acid, and it is therefore possible to achieve different tissue levels of AA[18]. The aim of the present work was to investigate whether it is possible to influence the accumulation of Cd in the organs of guinea pigs by different levels of AA intake.

## *Materials and methods*

Tricoloured male guinea pigs (Velaz, Prague) with an initial weight of 350–450 g were housed under standard laboratory conditions in plastic cages with wood chip bedding at 25 °C.

During a two-week adaption period, animals were fed a standard laboratory diet for guinea pigs (Mok Velaz, Prague) with the addition of vegetables. Two weeks later, the animals were fed an ascorbic acid-free diet: sugar 100 g/kg, oat flakes 490 g/kg, milk powder 300 g/kg, butter 100 g/kg, salt 10 g/kg. The guinea pigs were divided into four groups (eight animals in each group) according to the intake of Cd (in the form of $CdCl_2$; Merck) and ascorbic acid (Farmakon, Olomouc) in the drinking water. One control group had a low intake of AA: 2 mg/animal/24 h (− C, − Cd), and one a high intake: 100 mg/animal/24 h (+ C, − Cd). Animals in Cd-treated groups were given 1 mg Cd/animal/24 h and at the same time a low (− C, + Cd) or a high intake of AA (+ C, + Cd) at the same level as in the control groups. Access to water and diet was ad libitum at all times. During the experiment the animals were weighed weekly. Two studies were carried out, with different durations of Cd treatment, either short-term (5 weeks) or subchronic (12 weeks).

After overnight fasting, the guinea pigs were killed by decapitation and the liver, kidney and testes, and in week 12 also the heart and brain, were excised and rinsed in ice-cold saline solution. Tissue samples weighing approximately 1 g were stored at − 20 °C until they were analysed for Cd.

Dry mineralization of tissues was performed using the Apion device (Tessek). The mineralization is carried out by leading a superoxidative gas mixture into the containers with the samples. Duration of the heating to the drying temperature of 110 °C was 45 min, and drying lasted 60 min. The mineralization temperature of 400 °C was reached after 4-h heating and the duration of mineralization was set to 14 h. After the mineralization procedure the white ash was dissolved in 2 M $HNO_3$ and deionized water was added up to the volume of 25 ml.

Cadmium in organs was determined using a flame atomic absorption spectrophotometer (model PU 9400 X, Unicam Analytical Systems). The standard Perkin-Elmer procedure[19] and the STAT flame AAS technique[20] were used. The accuracy was verified by analysing Czechoslo-

vakian reference material: Bovine liver 12-2-01. The mean result obtained for 8 determinations was 0.493 ± 0.021 µg/g while certified Cd concentration is 0.480 ± 0.030 µg/g.

The results were evaluated statistically by the analysis of variance (ANOVA) and regression analysis (Statgraphics). The level of significance was set at $p < 0.05$.

*Results*

Body weights of the animals in both control groups increased during the experiment. In the Cd-treated groups the weight showed a tendency to increase up to the 5th week. Then it remained approximately constant (+ C, + Cd) or decreased slightly (− C, + Cd) so that at the end of the experiment the weight of animals in the Cd-treated groups was significantly lower than in the control groups (fig. 1). Cadmium accumulation in organs in different dietary groups is presented in the table. Compared with the control groups, the administration of Cd led to a manyfold increase of its levels in all investigated organs

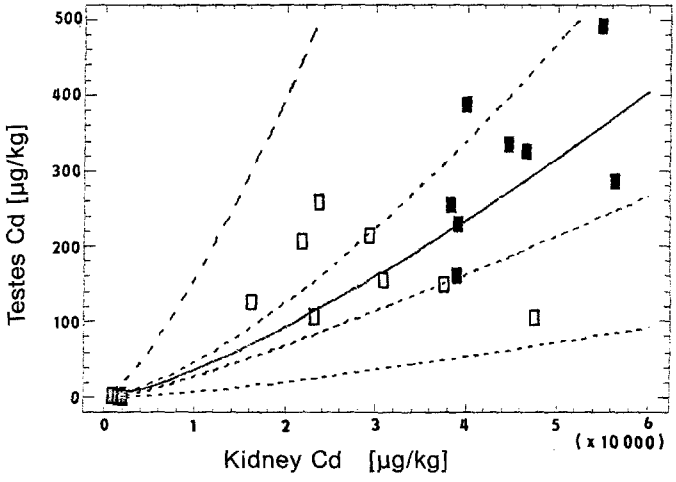


Figure 2. Correlation between cadmium concentrations in the kidneys and testes (multiplicative model, $r = 0.958$, $p < 0.0001$) in week 5 of the experiment. Confidence and prediction limits appear on the regression plot as the pair of dotted lines closest to and farthest from the regression line respectively. Data in the zero region represent Cd concentrations in the control groups. ■, − C, + Cd; □, + C, + Cd.

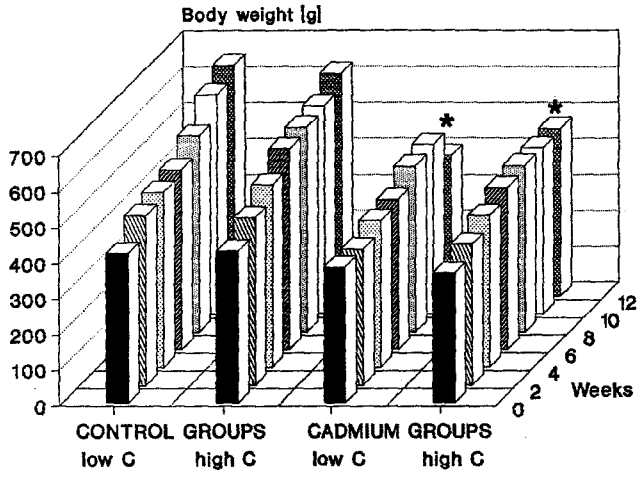


Figure 1. Influence of cadmium on body weight of male guinea pigs (* significantly different from control groups). Means for 8 animals.

in the following order: kidneys > liver > heart > testes > brain. In the control groups accumulation of Cd was not observed. At week 12 the concentrations of Cd in the liver, kidneys and testes were about twice those found after 5 weeks of exposure.

In the short-term experiment intake of high doses of AA statistically significantly decreased the levels of Cd in the kidneys and testes, by about 22% and 42% respectively. In the subchronic Cd-treatment experiment high doses of AA significantly decreased Cd accumulation in the testes, brain and heart by about 20%, 23% and 29% respectively. After 12 weeks, ascorbic acid lowered Cd concentration in the kidneys but the effect was not significant. Ascorbic acid had no effect on Cd accumulation in the liver. At weeks 5 and 12 we found a close correlation between the individual values in separate organs (correlation coefficients were in the range 0.890–0.990,

Cadmium levels in the organs of guinea pigs after short-term and subchronic cadmium treatment. Concentrations (mean ± standard error) are presented as mg/kg or µg/kg wet weight.

| Weeks of experiment | Groups | n | Kidneys [mg/kg] | Liver [mg/kg] | Testes [µg/kg] | Heart [µg/kg] | Brain [µg/kg] |
|---|---|---|---|---|---|---|---|
| 5 | − C, − Cd | 8 | 1.05 ± 0.12[a] | 0.22 ± 0.03[a] | ND | not analysed | not analysed |
| | + C, − Cd | 8 | 1.69 ± 0.11[a] | 0.35 ± 0.05[a] | ND | | |
| | − C, + Cd | 8 | 41.26 ± 2.67[c] | 15.83 ± 1.19[b] | 298.9 ± 38.8[b] | | |
| | + C, + Cd | 8 | 32.27 ± 4.98[b] | 16.11 ± 1.73[b] | 175.2 ± 24.3[a] | | |
| 12 | − C, − Cd | 8 | 1.79 ± 0.23[a] | 0.46 ± 0.04[a] | ND | ND | ND |
| | + C, − Cd | 8 | 1.10 ± 0.08[a] | 0.28 ± 0.04[a] | ND | ND | ND |
| | − C, + Cd | 8 | 99.59 ± 10.39[b] | 28.01 ± 2.24[b] | 595.9 ± 19.1[b] | 1646.3 ± 43.9[b] | 102.4 ± 5.9[b] |
| | + C, + Cd | 8 | 85.29 ± 8.52[b] | 34.12 ± 3.17[b] | 498.0 ± 16.2[a] | 1162.5 ± 87.1[a] | 78.9 ± 5.6[a] |

Groups: − C, − Cd: low vitamin C,
+ C, − Cd: high vitamin C,
− C, + Cd: low vitamin C + 10 mg Cd/l,
+ C, + Cd: high vitamin C + 10 mg Cd/l,

n – number of animals

a, b, c – different superscripts indicate singificantly different means ($p < 0.05$) in the same column for week 5 or week 12

ND – not detectable.

$p < 0.0001$). Figure 2 represents correlation between Cd levels in the kidneys and testes. The levels of Cd in the tissues of guinea pigs with suboptimal AA intake are shifted to the higher Cd concentration region.

*Discussion*

Mean intake of Cd in guinea pigs was 1 mg/24 h/animal, which exceeds by 15 times the maximal acceptable daily intake in humans permitted by FAO/WHO[21]. Our results indicate selective accumulation of Cd in certain organs after short-term and subchronic Cd-treatment. Concentration of Cd was highest in the kidneys and liver and the levels of Cd in these organs increased with the duration of exposure. The deposition of Cd especially in the liver and kidneys is the obvious result of its binding to metallothionein (MT), the formation of which is induced in these organs[22,23]. Kidneys are the critical organ of chronic Cd exposure. This can be partly explained by the transport of cadmium-metallothionein from the liver into the kidney[24]. Renal toxicity can be detected when a critical concentration of Cd is reached in the kidney[25].

After 12 weeks of exposure, which represents 6% of the life-span of guinea pigs, the concentration of Cd in the kidneys reached the value of 99.6 mg/kg. The critical level for man is 200 mg/kg[25]. However, for about 10% of the human population a Cd concentration in the kidneys lower than that considered critical may be dangerous[26].

Nutrients which decrease the toxicity of cadmium also decrease the uptake and retention of small amounts of dietary Cd[15]. Ascorbic acid is one of the nutrients which reduce the toxic effects of Cd and influence its accumulation. Data from animal experiments in which the effects of AA on the decrease of Cd accumulation in organs were investigated are not consistent[16,17,27,28]. The results of the present work are unambiguous in comparison with the results of experiments on rats[16,17,27]. This may be due to the use of guinea pigs as an experimental model. Guinea pigs, like man, are dependent on exogenous AA, and the tissue levels of ascorbic acid can therefore be regulated by the intake of AA from the diet.

The protective effect of AA against Cd accumulation and toxicity seems to depend upon several mechanisms. Ascorbic acid probably reduces the absorption of Cd from the gastrointestinal tract. Ascorbic acid greatly stimulates the absorption of iron from the intestines by reduction of ferric ions to ferrous ones. There is evident competition between Fe and Cd, which is shifted in favour of iron[16] by AA. A high intake of vitamin C elevates the concentration of MT in the tissues[29]. Metallothioneins, which are low molecular weight proteins, rich in sulfhydryl (SH) groups, are important for the transport of cadmium and prevention of its toxic effects. As a reducing agent, AA prevents the oxidation of SH-groups to non-functional disulfide groups. Ascorbic acid produces complexes with heavy metal cations[30]. It may be possible that AA decreases in this way the toxic effect of free Cd, or facilitates its elimination from the organism.

Our results are potentially important for the prevention of toxic effects of cadmium in smokers. It is obvious that besides food consumption the smoking of cigarettes is another main source of Cd. Levels of Cd in the blood and tissues of smokers are significantly higher in comparison with those of non-smokers, and simultaneously the degradation of AA in smokers is substantially increased[31]. Therefore it may be possible that an elevated intake of AA might, to a certain extent, decrease the toxic effects of Cd caused by smoking.


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