Effect of cadmium on hepatic microsomal monooxygenase activities in guinea pigs with low and high ascorbic acid intake

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The influence of in vivo administration of cadmium on hepatic microsomal cytochrome P-450 and cytochrome b_5 content, aniline hydroxylase, p-nitroanisole O-demethylase, and NADPH-cytochrome c reductase activities was investigated in guinea pigs with low (2 mg/animal/day) and high (100 mg/animal/day) ascorbic acid intake. Male guinea pigs received 10 mg Cd/L drinking water for 5 and 12 weeks. Cadmium administration resulted in significant decreases in cytochrome P-450 content and NADPH-cytochrome c reductase activity in the group of guinea pigs with low ascorbic acid intake, while the decreases were less evident in guinea pigs with high ascorbic acid intake. The activities of aniline hydroxylase and O-demethylase were not apparently changed by Cd administration, except for the group of guinea pigs with high ascorbic acid intake in 12th week of cadmium treatment, when increased activities were observed. These findings suggest that hepatotoxic effect of cadmium manifested by inhibition of hepatic microsomal metabolism may be in part beneficially moderated by enhanced ascorbic acid intake.
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Keywords: cadmium; cytochrome P-450; ascorbic acid; guinea pigs

Introduction

The effects of cadmium (Cd) on hepatic microsomal monooxygenase reactions in experimental animals have been extensively studied in in vivo experiments[1–8] and in vitro experiments using microsomal preparations.[1,8–10] Cadmium treatment decreased the rate of drug metabolism, with a marked decline in hepatic cytochrome P-450 content. Such effects of Cd on hepatic monooxygenases in vivo are caused by changes in heme metabolism. Cadmium affects the activity of enzymes responsible for the synthesis and degradation of heme, and dramatic increase in microsomal heme oxygenase activity was observed.[4,5]

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Another manifestation of Cd toxicity is a reduction of ascorbic acid (AA) concentration in the tissues.[11,12] Enhanced intake of AA by experimental animals had preventive and therapeutic effects on dietary Cd toxicity.[13–16] In guinea pigs the AA deficiency produced a marked decline in hydroxylation reactions, both cytochrome P-450 and cytochrome P-450 reductase activity, as well as in *N*- and *O*-dealkylation reactions.[17–20] These progressive changes depended on the degree of AA deficiency. Because AA concentration is reduced in the organism by Cd intoxication and Cd itself depresses hepatic cytochrome P-450 dependent monooxygenase activities, it could be assumed that suboptimal intake of AA may potentiate the toxic effects of Cd.

We therefore examined whether low and high ascorbic acid intake can affect cadmium-induced depression of hepatic microsomal cytochrome P-450–dependent monooxygenase activities in guinea pigs subchronically exposed to cadmium.

Methods and materials

Chemicals

D-glucose-6-phosphate monosodium salt, yeast D-glucose-6-phosphate dehydrogenase, NADP+, NADPH, NADH, and crystalline bovine serum albumin were purchased from Boehringer Mannheim (Mannheim, Germany). Aniline and cytochrome-c were obtained from Sigma Chemical Co. (St. Louis, MO USA), p-aminophenol, p-nitrophenol, and cadmium chloride were obtained from Merck (Darmstadt, Germany). AA was obtained from Farmacon (Olomouc, Czech Republic). All other chemicals were of analytical grade.

Animals

Male short-hair, three-colored guinea pigs (Velaz Prague) weighing 350 to 450 g were used in the experiment. After 2 weeks of feeding with standard laboratory diet with the addition of vegetables, the animals were randomly divided into four groups. Two control groups received AA in drinking water (control-low AA intake group; control-high AA intake group). AA content in drinking water, as well as consumption of drinking water, were monitored. The average dose of AA was calculated as follows: 2 mg of AA/animal/day for groups with low AA intake and 100 mg of AA/animal/day for groups with high AA intake. It is to be pointed out that daily consumption of drinking water in individual animals varied. The minimum requirement for AA for guinea pigs is 0.5 mg AA/animal/day. In this study, the dose of AA in the groups with low AA intake was four times higher, and we consider this intake of AA as suboptimal. High AA intake could be considered as the saturation dose. Two Cd-intoxicated groups were given Cd (as cadmium chloride) in drinking water at the concentration of 10 mg/Cd/L drinking water (Cd-low AA intake group; Cd-high AA intake group). AA intake in these groups of guinea pigs was the same as in the control groups. During the experiment the animals received drinking water and a vitamin C-free diet ad libitum. Composition of the AA-free diet was: sugar 100 g/kg, oat flakes 490 g/kg, milk powder 300 g/kg, butter 100 g/kg, salt 10 g/kg. The adequate amounts of other vitamins and minerals in the diet result from the fact that guinea pigs fed this diet supplemented with AA in drinking water showed no signs of a deficiency even after 1 year. The animals were decapitated after a 17-hour fast at the end of the 5th and 12th weeks of Cd administration. The number of decapitated guinea pigs in each group was eight.

Liver microsomes

The livers were quickly removed, weighed, chilled, and homogenized in ice-cold 0.15 M KCl containing 100 mM Tris-HCl and 10 mM EDTA pH = 7.4 using a Potter-Elvehjem glass homogenizer with Teflon pestle. The 20% (wt/vol) homogenate was centrifuged at 12,000*g* for 15 min and liver microsomes were obtained by centrifugation of supernatant fraction at 100,000*g* for 60 min. The microsomal pellet was resuspended in buffer (100 mM Tris buffer pH = 7.4 containing 1 mM EDTA and 30% glycerol) and stored at −80° C for 1 month.

Assays

In frozen liver microsomes protein concentration,[21] aniline hydroxylase activity,[22] p-nitroanisole *O*-demethylase activity,[23] NADPH-cytochrome C reductase activity,[24] and content of cytochromes P-450 and b_5[25] were determined. For the determination of cytochromes P-450 and b_5, a dual wavelength spectrophotometer UV/VIS Pye Unicam SP 8-100 (Pay Unicam, Cambridge, England) was used. NADPH-cytochrome c reductase activity was measured on a Hewlett-Packard 8452 A Diode Array Spectrophotometer (Hewlett-Packard, Palo Alto, CA USA).

Statistical analysis

Data are presented as means ± SEM and statistically analyzed by variance analysis (ANOVA, Statgraphics Manugistics, Inc., Maryland, USA). The acceptable level of significance was set at $P < 0.05$. Cd-treated groups with low and high AA intake were compared with the groups with low and high AA intake, which served as control groups.

Results

Table 1 and *Figure 2* show that high intake of AA for 5 and 12 weeks significantly increased the hepatic con-

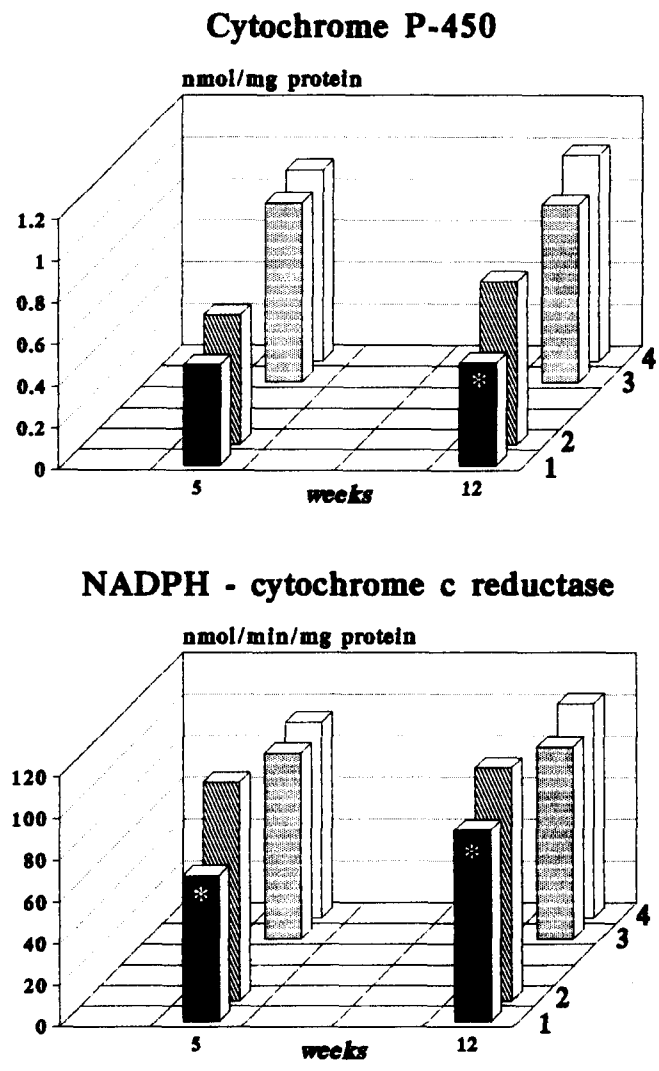

Figure 1 Effect of cadmium administration on cytochrome P-450 content and NADPH-cytochrome c reductase activity in liver microsomes of guinea pigs with low and high AA intake. Groups: 1–low AA intake + Cd; 2–low AA intake; 3–high AA intake + Cd; 4–high AA intake. *Significantly different from group 2; $P < 0.05$: Cytochrome P-450 significant differences (not designated above) are: group 1 to group 3 on 5th week ($P < 0.05$); group 1 to group 3 on 12th week ($P < 0.05$); group 2 to group 4 on 5th week ($P < 0.05$).

Table 1 Effect of cadmium on hepatic microsomal monooxygenase activities in guinea pigs with low and high intake of ascorbic acid

Weeks of experiment	Groups	n	Microsomal protein [mg/g liver]	Cytochrome P-450 [nmol/mg protein]	Cytochrome b_5 [nmol/mg protein]	Aniline hydroxylase [nmol/min/mg protein]	O-demethylase [nmol/min/mg protein]	NADPH-cytochrome c reductase [nmol/min/mg protein]
5	−AA, −Cd	8	17 ± 1[a]	0.62 ± 0.08[ab]	0.37 ± 0.02[ab]	0.77 ± 0.16[a]	0.90 ± 0.05[a]	105 ± 18[a]
	+AA, −Cd	8	18 ± 1[ab]	0.91 ± 0.08[c]	0.50 ± 0.05[c]	0.99 ± 0.05[a]	0.85 ± 0.08[a]	94 ± 6[ab]
	−AA, +Cd	8	22 ± 2[b]	0.48 ± 0.11[a]	0.30 ± 0.03[a]	0.71 ± 0.09[a]	0.78 ± 0.05[a]	70 ± 9[b]
	+AA, +Cd	8	20 ± 2[ab]	0.86 ± 0.05[bc]	0.42 ± 0.03[bc]	0.91 ± 0.07[a]	0.87 ± 0.09[a]	89 ± 10[ab]
12	−AA, −Cd	8	18 ± 2[ab]	0.78 ± 0.07[a]	0.51 ± 0.05[a]	0.69 ± 0.09[a]	0.71 ± 0.06[a]	112 ± 10[a]
	+AA, −Cd	8	19 ± 2[b]	0.99 ± 0.05[a]	0.59 ± 0.05[a]	0.79 ± 0.07[a]	0.75 ± 0.07[a]	103 ± 8[ab]
	−AA, +Cd	8	13 ± 1[a]	0.49 ± 0.05[b]	0.41 ± 0.06[a]	0.71 ± 0.14[a]	0.86 ± 0.09[b]	92 ± 4[b]
	+AA, +Cd	8	20 ± 2[b]	0.85 ± 0.09[a]	0.45 ± 0.02[a]	1.06 ± 0.14[b]	0.98 ± 0.08[b]	92 ± 5[b]

Groups: −AA, −Cd: low AA. +AA, −Cd: high AA. −AA, +Cd: low AA + 10 mg Cd/L. +AA, +Cd: high AA + 10 mg Cd/L.
Data represent mean ± SEM. n, number of animals.
[a,b,c]Different superscripts indicate significantly different means ($P < 0.05$) in the same column for week 5 or 12.

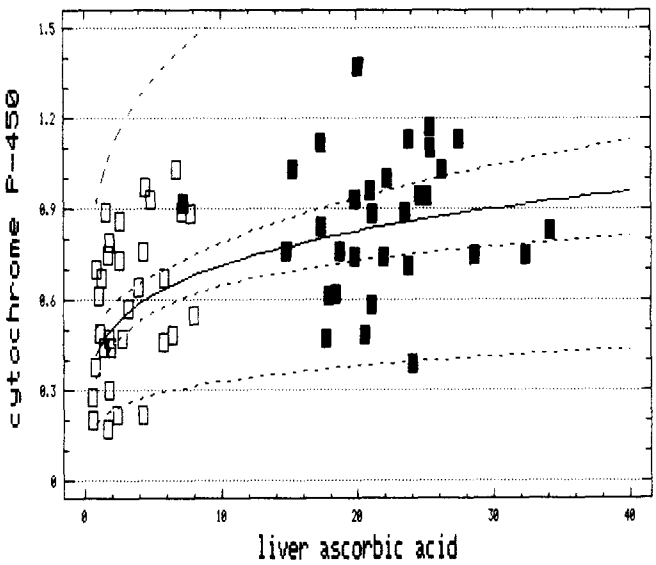

Figure 2 Correlation between AA concentration in liver and cytochrome P-450 in hepatic microsomes of guinea pigs exposed to cadmium (Multiplicative model; $r = 0.569$, $P < 0.0001$). Confidence limits appear on the regression plot as the pair of dotted lines closest to the regression line; prediction limits appear farthest from the regression line. Cytochrome P-450 content expressed as nmol/mg protein; data of liver AA concentration expressed as mg/100g, were taken from Mozesova and Ginter.[12] □ Groups with low AA intake; ■ groups with high AA intake.

tent of cytochrome P-450 in both Cd-treated and Cd-untreated guinea pigs. Similarly, high AA intake significantly increased the cytochrome b_5 content in liver microsomes in the group of guinea pigs in the 5th week of experiment (by 35% and 40%, respectively). High doses of AA significantly increased the microsomal protein concentration and aniline hydroxylase activity in Cd-treated guinea pigs at 12 weeks of experiment (by 49% and 54%, respectively), but the activities of *p*-nitroanisole *O*-demethylase, and NADPH-cytochrome c reductase were not apparently changed by high AA intake.

Cd administration significantly decreased the content of cytochrome P-450 in guinea pigs with low AA intake at the 12th week of the experiment, while the decrease was not significant in guinea pigs with high AA intake *(Figure 1)*. Cadmium treatment for 5 and 12 weeks tended to decrease the content of cytochrome b_5, but these differences were not significant. Activities of microsomal aniline hydroxylase and *p*-nitroanisole *O*-demethylase were not significantly affected by a 5-week Cd administration period. Unexpected results were obtained in the group of guinea pigs with high AA intake intoxicated with Cd for 12 weeks. Activities of aniline hydroxylase and *p*-nitroanisole *O*-demethylase in this group of guinea pigs increased significantly by Cd administration (by 34% and 31%, respectively). NADPH-cytochrome c reductase activity was significantly decreased by Cd administration for 5 and 12 weeks in the groups of guinea pigs with low AA intake (by 18% and 33%, respectively), while its activities were only slightly affected by cadmium in the group with high AA intake *(Figure 1)*.

Discussion

The present study demonstrates that subchronic administration of Cd in drinking water to guinea pigs for 5 and 12 weeks resulted in decreases in hepatic cytochrome P-450 content and NADPH-cytochrome c reductase activity predominately in the groups of guinea pigs with low AA intake. High AA intake prevented Cd-induced depression of cytochrome P-450 and inhibition of the reductase activity. Similar extent of the decrease in cytochrome P-450 content after Cd treatment was reported by other authors.[1,2,4,5,7] However, cadmium in these studies was administered intraperitoneally to male rats, which are capable of synthetizing vitamin C, in a single and relatively high dose. In contrast, oral administration of Cd in the diet for 15 days stimulated in rats the activity of hepatic detoxication enzymes.[26] The stimulation was dependent on the concentration of cadmium acetate in the diet (100 to 5000 ppm). Similarly, the lack of depression in cytochrome P-450 content in rats exposed to Cd in drinking water (100 ppm) for 24 weeks,[27] as well as no alteration in hepatic drug metabolism in rats chronically exposed to Cd in drinking water

(5 to 20 ppm) for 50 weeks,[6] were observed. These authors[6,27] suggested that in rats chronically exposed to sublethal doses of Cd, the lack of depression of hepatic cytochrome P-450 and drug metabolism can be a result of protective effects of high cellular levels of metallothionein, a cadmium-binding protein, synthetized in response to cadmium exposure. The effect of different routes, doses, or exposure time to cadmium could be the reason for these conflicting results.

In guinea pigs, which are dependent on exogenous AA intake, and which are in general more sensitive to the effects of xenobiotics, in vivo and in vitro studies have shown that cadmium significantly lowered the hepatic cytochrome P-450 level[8,9] and NADPH-cytochrome c reductase activity.[9] Our observations indicate that Cd in vivo significantly decreased the cytochrome P-450 content in guinea pigs with low AA intake, while AA supplementation protected them from depression of cytochrome P-450. In this respect, our results are different from the findings of the authors[8] who observed practically the same effect of Cd on hepatic cytochrome P-450 levels in AA deficient, sufficient, and excessive groups of guinea pigs. Cd had no effect on NADPH-cytochrome c reductase activity in rats.[1,10] However, the addition of Cd to microsomal suspension from guinea pig liver in vitro exerted a significant effect on the electron transfer components of microsomal monooxygenases.[9] Our results show the inhibition of microsomal NADPH-cytochrome c reductase activity in Cd-intoxicated guinea pigs and support the above mentioned observation from in vitro studies.

Inhibitory effects of Cd on microsomal aniline hydroxylase and *p*-nitroanisole *O*-demethylase activities reported for rats[1,2,5] were not observed in this study. Moreover, the activities of both enzymes increased significantly in guinea pigs with high AA intake on the 12th week of Cd administration.

It has been demonstrated that dietary nutrients such as Zn, Fe, Mn, Cu, Se, Ca, and AA alter the intestinal uptake and toxicity of Cd in animals.[13-16,28] Iron was the most effective mineral for preventing Cd accumulation in rats,[28] and the enhancement of Fe absorption in Cd-fed animals by combined administration of Fe and AA has been repeatedly reported.[14,16] The supplementation of Fe and AA in the diet prevented dietary cadmium toxicity[13] and a decrease of hematocrit and hemoglobin values in rats.[14] The recovery from anemia caused by cadmium was faster in rats given Fe and AA than in those on basal diet alone.[15] When AA was given alone, the absorption of Fe was not improved, whereas the Cd accumulation was significantly lowered.[28] The authors supposed that the improvement in Fe absorption was not the only effect of AA on the gastrointestinal uptake of Cd. High AA intake decreased Cd accumulation in this experiment also.[29]

The catabolism of cytochrome P-450 heme is associated with the stimulation of hepatic microsomal heme oxygenase,[30] the rate-limiting enzyme in heme degradation, which is very effectively induced by cadmium.[4,5] The increases of hepatic heme oxygenase activities were also observed in vitamin C-deficient guinea pigs.[31,32] The concepts about the mechanism of the stimulation of heme oxygenase are controversial. Accordingly, the increase in heme oxygenase activity might be produced directly by the absence of AA and cause the accelerated catabolism of cytochrome P-450 heme—or conversely—the increase of heme oxygenase activity might be a result of the accelerated catabolism of cytochrome P-450 in AA deficiency, whereby the free cytochrome P-450 heme acts as a stimulator of heme oxygenase.[31]

We suppose that stimulation of hepatic heme oxygenase by Cd and the following degradation of hepatic cytochrome P-450 heme, which could be enhanced by restriction of AA intake, are probably responsible for the decline of the cytochrome P-450–dependent monooxygenase activities in Cd-intoxicated guinea pigs with suboptimal AA intake. We conclude that supplementation of AA to guinea pigs may at least in part protect them from depression of hepatic cytochrome P-450–dependent monooxygenase activities induced by subchronic exposure to cadmium.

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