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Hepatic Monooxygenases and Conjugating Enzymes in Cadmium-intoxicated Hamsters: Effect of Vitamin C Supplementation

A. NAGYOVÁ[1], E. GINTER[1], M. ŠTEFEK[2], S. YANEV[3]

[1]*Institute of Preventive and Clinical Medicine,* [2]*Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic and*
[3]*Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

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Summary

The effects of cadmium and simultaneous administration of cadmium and vitamin C on hepatic microsomal monooxygenase activities, conjugation enzyme activities and enzyme activities in the serum were investigated in hamsters. Cadmium, as cadmium chloride, was administered to hamsters in a subtoxic dose in drinking water (10 mg Cd per liter) for 10 weeks. The majority of hepatic microsomal monooxygenases and enzyme activities in the serum reflecting liver damage were not significantly affected by subchronic cadmium treatment. On the other hand, cytosolic glutathione S-transferase and serum alanine aminotransferase were significantly changed by cadmium and these changes were effectively eliminated by the simultaneous administration of vitamin C (1 g per liter of drinking water). The results indicate that long-term supplementation with vitamin C may be effective in the protection of hepatic enzymes against cadmium toxicity.

Key words

Cytochrome P-450 – Cadmium – Vitamin C – Hamsters – Hepatic monooxygenases

Introduction

Cadmium (Cd) is a well-known contaminant of our environment and its hepatotoxic effects have been confirmed by several authors (Dudley *et al.* 1982, Goering and Klaassen 1984). In experimental animals, Cd elevates serum enzyme activities reflecting liver injury (Suzuki and Yoshida 1978, Khandelwal *et al.* 1991) and alters the activity of the hepatic cytochrome P-450-dependent monooxygenase system. The acute effect of Cd on hepatic monooxygenases significantly decreases hepatic cytochrome P-450 levels and monooxygenase activity (Hadley *et al.* 1974). In contrast, chronic exposure to Cd has been reported to have no effects on hepatic drug metabolism in rats (Schnell *et al.* 1978) and, moreover, stimulates hepatic detoxication enzymes (Wagstaff 1973).

The activity of the cytochrome P-450-dependent monooxygenase system depends on several factors (environmental, genetic, sex, age, etc.) and also on the vitamin C supply. An enhanced ascorbic acid (AA) intake increases the cytochrome P-450 content, O- and N-demethylase activities in a dose-dependent manner (Sato and Zannoni 1974, Ginter *et al.* 1984) and its protective effect on cadmium toxicity has been also reported (Fox *et al.* 1971, Suzuki and Yoshida 1978).

The aim of the present study was to investigate whether vitamin C supplementation in subchronically Cd-intoxicated hamsters may affect hepatic monooxygenase activities which are responsible for the metabolism of xenobiotics and physiologically important substances.

Methods

Animals

Male Syrian hamsters (VELAZ Prague) were used in the experiments. Animals were randomly divided

into three groups. The first group of hamsters served as control. The second group received cadmium (as cadmium chloride) in drinking water at the concentration of 10 mg of cadmium per liter. The third group of hamsters was concomitantly treated with cadmium in the same dose in drinking water as the second group and ascorbic acid (1 g AA, 1 g per liter of drinking water). During the whole experiment the animals received drinking water and standard laboratory diet *ad libitum*. Animals were decapitated after fasting for 15 h in the 10th week of cadmium and ascorbic acid (vitamin C) administration.

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 at pH 7.4, using a Potter-Elvehjem glass homogeniser with a teflon pestle. The 20 % (w/v) homogenate was centrifuged at 12 000 x *g* for 15 min and liver microsomes were obtained by centrifugation of supernatant fraction at 100 000 x *g* for 60 min. The microsomal pelet was resuspended in a freeze buffer (100 mM Tris buffer at pH 7.4, containing 1 mM EDTA and 30 % glycerol) and stored at −80 °C before use.

Enzyme activity assays

In liver microsomes, protein and haeme concentrations (Lowry *et al.* 1951, Paul *et al.* 1953), aniline hydroxylase (Holtzman and Gillette 1969), p-nitroanisole O-demethylase activity (Netter and Seidel 1964), ethylmorphine N-demethylase (Nash 1953), ethoxycoumarin O-deethylase (Aitio 1978), NADPH-cytochrome c reductase (Vermilon and Coon 1978), UDP-glucuronyl transferase, EC 2.4.1.17 (Frei *et al.* 1970) activities and the content of cytochromes P-450 and b_5 (Omura and Sato 1964) were determined. For assessing cytochromes P-450 and b_5 a dual wavelength spectrophotometer UV/VIS Pye Unicam SP 8-100 was used. NADPH-cytochrome c reductase activity was measured on a Hewlett-Packard, Model 8452 A diode array spectrophotometer and ethoxycoumarin O-deethylase activity was estimated using a Perkin-Elmer fluorescence spectrophotometer Model LS-5. Cytosolic glutathione S-transferase activity toward the aryl substrate (CDNB), EC 2.5.1.13, was assessed as described by Baars *et al.* (1978). Enzyme activities in blood serum: alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1) and gamma-glutamyl transferase (GGT, EC 2.3.2.2) were determined using commercial biochemical kits (Biola-test, Lachema, Brno).

Statistical analysis

Data are presented as means ± S.E.M. and statistically analyzed by variance analysis (ANOVA, Statgraphics). Values for $p<0.05$ were considered to be significant.

Table 1
Effect of cadmium and vitamin C administration on hepatic monooxygenase activities of hamsters *

Parameter	Control	Cd-intoxicated	Cd-intoxicated + vitamin C
Liver Cd levels (mg/kg)	1.66 ± 0.14	45.76 ± 3.26[a]	75.13 ± 4.92[a,b]
Microsomal protein (mg/g liver)	19 ± 1	19 ± 1	16 ± 1
Microsomal haeme (nmol/mg)	1.61 ± 0.11	1.64 ± 0.11	2.22 ± 0.16[a,b]
Cytochrome P-450 (nmol/mg)	0.72 ± 0.03	0.66 ± 0.06	0.99 ± 0.08[a,b]
Cytochrome b_5 (nmol/mg)	0.19 ± 0.01	0.19 ± 0.02	0.23 ± 0.02
p-nitroanisole O-demethylase (nmol/mg/min)	0.65 ± 0.06	0.82 ± 0.05	0.92 ± 0.04[a]
Ethylmorphine N-demethylase (nmol/mg/min)	2.84 ± 0.17	2.76 ± 0.39	2.86 ± 0.12
Aniline hydroxylase (nmol/mg/min)	0.63 ± 0.05	0.69 ± 0.05	0.86 ± 0.03[a,b]
Ethoxycoumarine O-deethylase (nmol/mg/min)	0.96 ± 0.10	1.02 ± 0.09	1.31 ± 0.11
NADPH-cytochrome c reductase (nmol/mg/min)	61 ± 4	79 ± 5[a]	86 ± 4[a]

* *Values, expressed per mg of microsomal protein, are means ± S.E.M. of 8 animals*
[a] *Statistically significant from control group ($p<0.05$)*
[b] *Statistically significant from Cd-intoxicated group ($p<0.05$)*

Results

Effect of cadmium and vitamin C on hepatic monooxygenase activities

The results are summarized in Table 1. The concentrations of microsomal protein and haeme were not significantly altered in the livers of Cd-intoxicated hamsters. Changes in total contents of cytochrome P-450 and cytochrome b_5 in liver microsomes from Cd-intoxicated hamsters were also not evident. Similarly, the activities of microsomal p-nitroanisole O-demethylase, ethylmorphine N-demethylase, aniline hydroxylase and ethoxycoumarin O-deethylase were not significantly affected in Cd-intoxicated hamsters; only NADPH-cytochrome c reductase activity was significantly higher (by 30 %). Simultaneous administration of cadmium and vitamin C significantly increased the haeme concentration (by 35 %), cytochrome P-450 content (by 50%) and aniline hydroxylase activity (by 25 %) in liver microsomes and these compared with the Cd-intoxicated group. The changes in cytochrome b_5 content (by 21 %) and ethoxycoumarin O-deethylase (by 28 %) were not significant.

Table 2
Effect of cadmium and vitamin C administration on hepatic conjugation enzyme activities of hamsters *

Parameter	Control	Cd-intoxicated	Cd-intoxicated + vitamin C
Cytosolic protein (mg/g liver)	80±9	110±7	135±4[a]
UDP-glucuronyl transferase (nmol/mg/min)	2.44±0.19	2.74±0.11	3.46±0.14[a,b]
Glutathione S-transferase (μmol/mg/min)	1.27±0.14	0.80±0.08[a]	1.15±0.09[b]

** Values, expressed per mg of protein, are means ± S.E.M. of 8 animals*
[a]Statistically significant from control group ($p<0.05$)
[b]Statistically significant from Cd-intoxicated group ($p<0.05$)

Table 3
Effect of cadmium and vitamin C administration on enzyme activities in the serum of hamsters *

Parameter	Control	Cd-intoxicated	Cd-intoxicated + vitamin C
Alanine aminotransferase (μkat/l)	0.56±0.03	0.85±0.07[a]	0.57±0.03[b]
Aspartate aminotransferase (μkat/l)	0.54±0.02	0.60±0.04	0.51±0.03
Alkaline phosphatase (μkat/l)	1.75±0.10	1.43±0.11	1.44±0.15
Gamma-glutamyl transferase (μkat/l)	0.19±0.02	0.20±0.04	0.24±0.08

** Values are means ±S.E.M. of 8 animals*
[a]Statistically significant from control group ($p<0.05$)
[b]Statistically significant from Cd-intoxicated group ($p<0.05$)

Effect of cadmium and vitamin C on hepatic conjugation enzyme activities

Cadmium administration increased cytosolic protein concentration by 24 % compared with control values but this difference was not significant (Table 2). The activity of UDP-glucuronyl transferase in liver microsomes was not changed in Cd-intoxicated hamsters, while cytosolic glutathione S-transferase activity towards the aryl substrate (CDNB) was significantly decreased (by 37 %). Vitamin C administration significantly increased the cytosolic protein concentration in Cd-intoxicated hamsters compared with control values (by 52 %). The activities of both conjugating enzymes, UDP-glucuronyl transferase and glutathione S-transferase, were also significantly increased by vitamin C supplementation (by 26 % and 44 %, respectively).

Effect of cadmium and vitamin C on enzyme activities in the serum

Four enzymes in the blood serum reflecting liver injury were measured in cadmium and vitamin C treated hamsters (Table 3). Cadmium significantly elevated the activity of alanine aminotransferase (by 52 %) while the activities of aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase were not significantly affected by cadmium treatment. Supplementation with vitamin C returned alanine aminotransferase activity, elevated by cadmium, to control values.

Discussion

Subchronic reatment of hamsters with cadmium had no appreciable effect on the activity of hepatic monooxygenases investigated in this study. Only NADPH-cytochrome c reductase activity was significantly increased compared with the control group. Similarly, no alterations in hepatic drug metabolism and cytochrome P-450 content were observed in rats chronically exposed to Cd in drinking water (Schnell *et al.* 1978 Kotsonis and Klaassen 1978). Moreover, stimulation of hepatic detoxication enzymes by dietary cadmium acetate was reported by Wagstaff (1973). In Japanese quails, the administration of 100 ppm cadmium in the diet for 45 days did not change hepatic cytochrome P-450 levels, but cytochrome c reductase activities increased in the Cd-treated group (Leonzio *et al.* 1992). The above mentioned authors suggested that the lack of depression of cytochrome P-450 during long-term exposure to cadmium is due to the induction of protective Cd-metallothionein (Schnell *et al.* 1978).

In contrast, cadmium is a potent inhibitor of hepatic microsomal metabolism in acute-exposure experiments (Hadley *et al.* 1974, Schnell *et al.* 1978). Here, depression of cytochrome P-450 and the concomitant decrease in monooxygenase activity is associated with Cd-induced microsomal haeme oxygenase, an enzyme which catalyzes haeme degradation (Krasny and Holbrook 1977).

Subchronic administration of Cd to hamsters did not affect the microsomal cytochrome P-450 content and haeme concentration in this study. However, in Cd-intoxicated hamsters simultaneous administration of vitamin C significantly increased both haeme and cytochrome P-450 concentrations. These data could support the view according to which ascorbic acid (AA) may affect haeme and cytochrome P-450 degradation (Omaye and Turnbull 1980).

The results of the present study indicate that cytosolic glutathione S-transferase was inhibited by chronic Cd exposure, but the decrease was overcome in hamsters supplemented with vitamin C. Contradictory results concerning the effects of Cd on GSH-conjugating enzymes were obtained in different animal species (Siegers *et al.* 1987, Freundt and Ibrahim 1991).

The protective effect of vitamin C in chronic Cd toxicity has also been documented. The beneficial effect of AA upon anaemia has been reported in young coturnix (Fox *et al.* 1971). Cadmium hepatotoxicity leading to increased serum aminotransferase activity, was prevented in rats by supplementing the diet with iron and AA (Suzuki and Yoshida 1978). Similar results were obtained in the present study, where supplementation with vitamin C returned alanine aminotransferase activity elevated by cadmium to control values.

When comparing the activities of aniline hydroxylase, UDP-glucuronyl transferase and cytochrome P-450 content of the group of Cd-intoxicated hamsters to the group receiving Cd and vitamin C, it seems that the increases are the results of vitamin C influence. This supports the observations of other authors about the stimulating effect of AA on the hepatic cytochrome P-450 dependent monooxygenase system (Ginter 1989).

It was demonstrated that AA induces metallothionein, a Cd-binding protein, in the mouse liver and that acute Cd toxicity can be prevented by the pre-injection of AA (Onosaka *et al.* 1987). These authors also observed that the Cd level in the liver of mice pre-injected with AA was higher than that of the controls. We observed a significant increase of cytosolic protein concentrations (Table 2) in Cd-intoxicated hamsters supplemented with vitamin C. These animals also had significantly higher Cd levels in the liver as compared with hamsters treated only with Cd (Table 1). We suppose that the increases of both cytosolic proteins and Cd levels may be associated with the increase of metallothionein induction in the liver of vitamin C supplemented hamsters.

In summary, the simultaneous administration of vitamin C effectively prevented Cd-induced changes in cytosolic glutathione S-transferase and serum alanine aminotransferase activities in subchronically Cd-intoxicated hamsters. Long-term supplementation with vitamin C may be effective in the protection of hepatic enzymes against cadmium toxicity.

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Reprint Requests

A. Nagyová, Ph.D., Institute of Preventive and Clinical Medcine, 833 01 Bratislava, Limbová 14, Slovak Republic.