INFLUENCE OF ACUTE CADMIUM EXPOSURE ON PLASMA ANTIOXIDANT PARAMETERS IN RATS

EMÍLIA HIJOVÁ, FRANTIŠEK NIŠTIAR* AND MILAN KUCHTA

Institute of Experimental Medicine, *Department of Pathological Physiology, Medical Faculty of Safarik University, 040 01 Košice, Slovakia
E-mail: hijova@pobox.sk

Received for publication September 17, 2003.

Abstract

The effects of acute cadmium exposure on parameters of antioxidant status was investigated in male and female Wistar albino rats. Administration of cadmium in single lethal dose (LD50 of CdCl2) or in this dose divided into three equal doses significantly (P<0.001) decreased the plasma total antioxidant status. The concentration of vitamin E was also significantly reduced (P<0.001). Similar tendency had concentration of uric acid. The mild non-significant decrease in vitamin C and zinc the concentrations was detected. These results show that the acute cadmium intoxication has an unfavourable effect on plasma antioxidant status in rats because of the consumption of extracellular antioxidants.

Key words: rats, cadmium, plasma antioxidant status, vitamin C, vitamin E, zinc, uric acid.

Material and Methods

Experimental animals. Male and female Wistar albino rats (n=36) aged 99 d of average weight 285.7±48.39 g were housed in conventional conditions on normal laboratory diet and supplied with drinking water. Animals were divided into three experimental groups (6 males and 6 females per group).

The first group (group 1) received LD50 of CdCl2 as a single dose applied by a stomach tube. The second group (group 2) received in the same way LD50 of CdCl2 divided into three doses (1/3 of LD50 daily) during three consecutive days. The third group (group 3 or control group) received drinking water without CdCl2.

Cadmium was applied between 6:00-7:00 a.m. in drinking water (1 ml/100 g b.w.). The LD50 value of Cd as CdCl2 per os for rats was 225 mg.kg⁻¹ (7). The intake of drinking water and food was controlled during the experiment.

After 24 h (group 1) or 72 h (group 2) the rats were anaesthetized (sodium pentobarbital, Pentobarbital Spofa, 50 mg.kg⁻¹ i.p.) and blood samples were taken from the heart by puncture using heparin (5000 IU.l⁻¹ inj.) as an anticoagulant. The samples were centrifuged at 1500 g for 15 min and the plasma samples were used for the determination of antioxidant parameters.

Antioxidant parameters. The plasma total antioxidant status was determined by a spectrophotometric method with the RANDOX kit (Total antioxidant status, Randox laboratories, UK). The measurement was carried out in an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland). The concentration of vitamin E was determined by HPLC method according to Sanz and Santa-Cruz (12) and that of vitamin C by colorimetric method according to Roe and Kuether (11). The plasma concentration of zinc and uric acid were measured using commercial kits (WAKO Chemicals GmbH, DE, and Lachema, Czech Republic, respectively).

Statistical analysis. Statistical analysis was performed by Student’s t-test and ANOVA to determine the significance. Statistical significance was accepted at P<0.05.
**Results**

In the course of acute cadmium exposure 4 females and 1 males from group 1 and 4 males and 2 females from group 2 died. During the experiment the animals were anxious, had accelerated breathing and visible bloody discharge from nostril and eyes. The internal organs were macroscopically enlarged and markedly congested.

The intake of food and water was decreased in both groups in comparison to the control group. The body weight was significantly decreased in group 1 from the initial weight of 317.1 ± 48.21 g to 296.4 ± 47.49 g (P<0.01) in the end of the experiment and in group 2 from 265.0 ± 48.48 g to 242.5 ± 44.47 g (P<0.01). No significant differences of body weight in control group were observed (275.0 ± 46.22 g vs 280.0 ± 51.52 g).

Changes in antioxidant parameters during acute cadmium exposure are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol/L)</td>
<td>1.21 ± 0.13</td>
<td>0.73 ± 0.08***</td>
<td>0.93 ± 0.11***</td>
</tr>
<tr>
<td>Vitamin C (µmol/L)</td>
<td>50.22 ± 10.98</td>
<td>39.31 ± 13.41</td>
<td>42.29 ± 14.60</td>
</tr>
<tr>
<td>Vitamin E (µmol/L)</td>
<td>8.38 ± 3.69</td>
<td>3.84 ± 0.73***</td>
<td>4.83 ± 1.39**</td>
</tr>
<tr>
<td>Zinc (µmol/L)</td>
<td>16.02 ± 3.38</td>
<td>15.92 ± 3.21</td>
<td>18.66 ± 1.77*</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>45.0 ± 7.93</td>
<td>33.0 ± 6.78***</td>
<td>36.16 ± 9.70*</td>
</tr>
</tbody>
</table>

The value are means ± SD. Significant differences were calculated by ANOVA, *P<0.05; ** P<0.01; ***P<0.001. The value are significantly different from those in the control group.

**Discussion**

Environmental contamination with different agents is recognized as a world-wide problem. Various possible mechanisms have been suggested to explain the damage induced by heavy metals. Proteins are major targets of damage by metals and the loss of protein function is usually a consequence of protein modification by metals. Metals have a special affinity toward (-SH) groups of proteins. By covalent binding to (-SH) groups, metals can block the functional sites of the catalytic or binding domains of enzymes or modify protein conformation.

The second possible mechanism may be the displacement of a metal, which is essential for biological activity of a molecule by another one. Most frequently, zinc-requiring enzymes are inactivated through direct displacement of Zn by another metal ion from the binding site. Transition metals are known to be able to generate extremely reactive oxygen species. A number of items have focused their attention on the influence of chronic cadmium intoxication on the activity of intracellular antioxidants, but measurement of total antioxidant status as an integrated marker of extracellular antioxidants after metal intoxications are rare. Acute exposure to cadmium can cause a variety of adverse effects for the health status. It was demonstrated that Cd is a potent inducer of the cell oxidative stress and affects antioxidant defence potential biphasically by the inhibition and enhancement of several antioxidant enzymatic and non-enzymatic molecule activity (4, 13).

In our experiment the significantly decreased values of TAS (P<0.001) in both groups after acute cadmium exposure could be an answer of plasma antioxidants to an elevation of reactive oxygen species. Vitamin C and vitamin E together constitute only 12% of the TAS in comparison with uric acid, which constitutes 33% of the TAS (8).

Cadmium is a nephrotoxic metal (3). The proximal tubules of the kidneys are a major target of chronic cadmium-induced toxicity. The development of cadmium-induced lesions in the kidneys is characterized by proteinuria and excessive urinary excretion of other substrates such as enzymes, amino acids, and glucose (3, 16). Exposure of renal cells to cadmium causes apoptotic features, DNA fragmentation, and chromatin condensation in earlier stages of cadmium cytotoxicity before the cadmium-induced necrotic phase (5). During acute cadmium intoxication the concentration of uric acid was significantly reduced in group 1 (P<0.001) and in group 2 (P<0.01). The observed hypouricaemia could be caused by a decreased uric acid synthesis or increased excretion of uric acid by the kidneys.

One important antioxidant in blood plasma and tissues with a very wide spectrum of biological effect is ascorbic acid (vitamin C). Ascorbic acid is produced from the ultimate hexose precursor D-glucose. After the pathway of ascorbic acid biosynthesis had been
established, it was soon revealed that in tissues of humans, monkeys and guinea pigs, there is no activity of the terminal enzyme of the pathway, L-gulono-γ-lactone oxidase (GLO) (9). Mice, rats and rabbits synthesize vitamin C in the liver. Kostic et al. (6) have observed elevated plasma concentration of ascorbate and tocopherol as a biological response to chronic cadmium chloride intoxication. During acute cadmium intoxication no significant changes of vitamin C were observed. It seems that changes in the concentration of vitamin C are time dependent because in response to Cd intoxication the rats in group 2 began to produce more vitamin C in comparison to rats in group 1. Contrary to their results Shukla and Chandra (14) administered cadmium (0.4 mg.kg\textsuperscript{-1}.day\textsuperscript{-1}) induced a significant decline in plasma tocopherol after 30 d. In our experiment, the concentration of vitamin E was significantly reduced in group 1 (P<0.001) and in group 2 (P<0.01).

The fact that zinc clearly plays a major role in the toxicity of cadmium may well be due to the similar chemical nature of both cadmium and zinc and their common interactions within living systems. This similar chemistry, combined with the greater affinity of cadmium for various bioligands, probably allows to displace zinc in many biological processes. Zinc as an antagonist of cadmium was nonsignificantly decreased after 24 h (group 1) from application of cadmium, but after 72 h (group 2) its concentration was increased (P<0.05).

From the present results it can be concluded, that acute cadmium intoxication caused a significant reduction of the plasma total antioxidant status and individual extracellular antioxidants in rats.

Acknowledgments. This work was supported by a grant VEGA 1/8235/01 from the Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences.

References