The Effects of Selenium on the Antioxidant Defense System in the Liver of Rats Exposed to Cadmium

B. OGNJANOVIC, R.V. ŽIKIĆ, A. ŠTAJN, Z.S. SAICIĆ 1
M.M. KOSTIC2, V.M. PETROVIĆ1

Faculty of Science, University of Kragujevac, Kragujevac, Serbia, 1Department of Physiology, Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia and 2Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia, Yugoslavia

Summary
Total superoxide dismutase (total SOD), copper zinc containing superoxide dismutase (CuZn SOD), and manganese superoxide dismutase (Mn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) activities as well as ascorbic acid (AsA), and vitamin E (vit E) concentrations were analysed in the liver of rats exposed to cadmium (15 mg Cd/day/kg), selenium (7 μg Se/day/kg), and to cadmium + selenium (15 mg Cd + 7 μg Se/day/kg), and in control animals. Cadmium caused a decrease of total SOD, Mn SOD, CAT and GSH-Px but an increase of GST activity in the liver of rats. Contrary to cadmium, selenium caused a significant increase of the activity of these enzymes except for GSH-Px. By concomitant exposure to both cadmium and selenium, the toxic effects of cadmium on the activity of mentioned enzymes were abolished. In all exposed groups, the activity of enzyme glutathione-S-transferase was enhanced, indicating its increased role in prevention of lipid peroxidation. Cadmium decreased the concentration of AsA and increased the concentration of vitamin E in the liver, while selenium increased the concentration of both vitamins. However, by concomitant administration of cadmium and selenium, these changes were diminished and tended to reach control values.

Key words
Cadmium - Selenium - Rat - Antioxidant defense system - Liver

Introduction
Cadmium is a very frequently present toxic agent in the environment. In the living organism, it causes significant metabolic changes and exerts deleterious effects upon biological systems. From the total amount of food cadmium intake, only about 6% is absorbed in the gastrointestinal tract of animals (Cherian 1980). The largest amounts of this metal (75%) are deposited in the liver and kidneys (Marafante 1976). However, the toxic activity of cadmium is exhibited when it is "free", i.e. when it is not bound to proteins (Webb 1979). In the living organisms cadmium causes anaemia (Kostić et al. 1993), decreased body growth (Chatterjee et al. 1973), injuries of the liver, kidneys and other organs (Kopp et al. 1983, Bomhard et al. 1984), decalcification of the skeleton, haemorrhagic lesions of the CNS and other tissue changes (Friberg et al. 1974, Samarawickrama 1979). Cadmium also influences the membrane transport (Verbost et al. 1989) and causes alterations in metabolism of carbohydrates, proteins and lipids (Piscator and Axelsson 1970, Rajanna et al. 1984, Dudley et al. 1984). Cadmium inhibits the activity of enzymes by binding to their sulphydryl groups, consequently causing the peroxidative destruction of cell membranes (Chung and Maines 1987). Moreover, cadmium decreases ascorbic acid concentration (Patnaik 1971), enhances the production of superoxide anion radicals (O2−) (Amoruso et al. 1982), increases lipid peroxidation (Jamall and Smith 1985c) and inhibits the activities of antioxidant defense system

Selenium is an essential trace element in the diet of animals, but in higher concentrations it becomes toxic and even lethal (Rosenfeld and Beath 1964). Selenium becomes a component of molecules of the selenium-dependent glutathione peroxidase (GSH-Px). It is also known that selenium plays a protective role against toxic actions of cadmium and other heavy metals (Bozkurt and Smith 1981, Chung and Maines 1987) because it alters the distribution of cadmium in tissues (Gasiewicz and Smith 1976) and induces binding of the Cd-Se complex to proteins which are similar to metallothioneins.

In the present experiments, the influence of cadmium and selenium on the antioxidant defense system was analysed in the rats liver. After 30 days of exposure, the activities of enzymatic (total SOD, CuZn SOD, Mn SOD, CAT, GSH-Px and GST) and nonenzymatic (ascorbic acid, vitamin E) components of this system were determined. The possible protective role of selenium against the toxic effects of cadmium has been especially considered.

Methods

Wistar albino male 60-day-old rats (weighing 200 ± 20 g at the beginning of experiments) were used. The animals were kept at 21 ± 2 °C, fed a pellet rat diet, and exposed to 12 h light-dark cycle. Control rats drank tap water ad libitum. The experimental groups drank water containing 200 μg CdCl₂ ml (15 mg Cd/day/kg), or 100 μg Na₂SeO₃/l (7 μg Se/day/kg) or water containing both cadmium and selenium for 30 days. The exposed rats were housed in individual cages with food and water ad libitum. Each experimental group consisted of six animals.

The animals were sacrificed by decapitation between 0800 and 1000h. Fresh heparinized blood was collected. The liver tissue was dissected out within 3 min, placed in ice-cold 155 mM NaCl and washed with the same solution. The liver tissue was then minced and homogenized in 10 volumes of 25 mM sucrose containing 10 mM Tris-HCl, pH 7.5 at 1500 rpm using a Thomas Sci Co. glass homogenizer (Teflon pestle), 8–10 up-and-down strokes. Homogenates were then centrifuged at 4 °C at 100 000 × g for 90 min.

Total SOD activity was assayed in the supernatant by the epinephrine method (Misra and Fridovich 1972). For the determination of Mn SOD activity the assay was conducted after preincubation with 8 mM KCN. Catalase was measured as described by Beutler (1982). The activity of glutathione peroxidase (GSH-Px) was assayed by following the oxidation of NADPH at 340 nm with t-butylhydroperoxide (Tamura et al. 1982). Glutathione-S-transferase (GST) activity toward l-chloro-2,4-dinitrobenzene as substrate was determined according to Habig et al. (1974). All enzyme assays were performed at 25 °C. Enzyme activities were expressed per g of wet mass (U/g w.m.). The ascorbic acid (AsA) content was measured by the dinitrophenyl-hydrazine method (Roe 1957) and vitamin E (vit E) by the method suggested by Desai (1984). The hemoglobin concentration in erythrocytes was estimated by the cyanmethaemoglobin method (Drabkin and Austin 1935) and the blood glucose concentration by the ortho-toluidine method (Hultmann 1959).

Cadmium and selenium in the liver were determined in damaged tissue using a mixture of nitric and perchloric acids (Shirley et al. 1949). Cadmium was determined by atomic absorption spectrophotometry while the concentration of selenium was determined by the fluorimetric method (Dye et al. 1960) using 3'3'-diaminobenzidine.

The Student's t test was used for data comparison between different groups (Hoel 1966).

Table 1
Growth of body mass (GBM) after 30 days, liver mass, relative liver mass, cadmium and selenium concentration in liver of rats exposed to cadmium (Cd), selenium (Se) and cadmium + selenium (Cd + Se) compared to controls (C)

<table>
<thead>
<tr>
<th></th>
<th>GBM %</th>
<th>Liver mass g</th>
<th>Relative liver mass g/100 g</th>
<th>Cd μg/g w.m.</th>
<th>Se μg/g w.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>100.0</td>
<td>18.1 ± 0.4</td>
<td>3.95 ± 0.12</td>
<td>0.3 ± 0.005</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>Cd</td>
<td>35.5***</td>
<td>12.6 ± 0.4****</td>
<td>3.52 ± 0.11*</td>
<td>21.3 ± 1.9****</td>
<td>0.82 ± 0.03*</td>
</tr>
<tr>
<td>Se</td>
<td>109.0</td>
<td>18.4 ± 0.4</td>
<td>3.78 ± 0.08</td>
<td>0.3 ± 0.002</td>
<td>1.81 ± 0.06****</td>
</tr>
<tr>
<td>Cd + Se</td>
<td>82.5**</td>
<td>14.4 ± 0.6***</td>
<td>3.62 ± 0.08</td>
<td>24.3 ± 0.3****</td>
<td>1.43 ± 0.06****</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 6 animals in each group. Significantly different from controls. *p < 0.05; **p < 0.02; ***p < 0.01 and ****p < 0.005
Results

Table 1 shows the data on the growth of body mass after 30 days of exposure, the absolute and relative liver mass as well as the cadmium and selenium concentrations in the liver. The growth of body mass of rats exposed to cadmium was significantly lower (p<0.005) with respect to controls. In rats exposed concomitantly to both cadmium and selenium, the growth of body mass was also significantly lower than in control rats (p<0.02). The liver mass decreased in animals exposed to cadmium (p<0.005), and in animals exposed to cadmium and selenium concomitantly (p<0.01), but it was not changed in rats exposed to selenium only. The relative liver mass was significantly decreased in rats exposed to cadmium (p<0.05), while it was not significantly changed in other experimental groups. The concentration of cadmium in the liver was significantly increased in animals exposed to cadmium and in animals exposed to cadmium and selenium concomitantly (p<0.005). Concentration of selenium was increased after exposure to cadmium (p<0.05), or to selenium (p<0.005), as well as after the concomitant exposure of rats to both cadmium and selenium (p<0.005).

Table 2
Haemoglobin and glucose concentration in the blood of rats after exposure to cadmium (Cd), selenium (Se) and cadmium + selenium (Cd+Se) compared with the controls (C).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cd</th>
<th>Se</th>
<th>Cd+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (mM/l)</td>
<td>9.20±0.03</td>
<td>7.82±0.08****</td>
<td>8.92±0.13</td>
<td>8.78±0.14**</td>
</tr>
<tr>
<td>Glucose (mM/l)</td>
<td>5.18±0.10</td>
<td>5.85±0.10**</td>
<td>5.30±0.12</td>
<td>5.04±0.08</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 6 animals in each group. Significantly different from controls. **p<0.02 and ****p<0.005

Table 2 depicts the results on haemoglobin and glucose concentrations in the blood. The haemoglobin concentration was decreased (p<0.005), but that of glucose was increased (p<0.02) in the cadmium-exposed rats. In the other treated groups, blood glucose was not significantly changed. In rats exposed to selenium the haemoglobin concentration was not appreciably altered, while concomitant exposure of rats to both cadmium and selenium diminished the toxic effects of cadmium.

Table 3
The activities of total SOD, CuZn SOD and Mn SOD in the liver of rats after exposure to cadmium (Cd), selenium (Se), and cadmium + selenium (Cd + Se) compared with the controls (C).

<table>
<thead>
<tr>
<th></th>
<th>Total SOD (U/g w.m.)</th>
<th>CuZn SOD (U/g w.m.)</th>
<th>Mn SOD (U/g w.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>6509±90***</td>
<td>5356±153</td>
<td>1139±40</td>
</tr>
<tr>
<td>Cd</td>
<td>6015±83**</td>
<td>5140±111</td>
<td>869±48**</td>
</tr>
<tr>
<td>Se</td>
<td>7386±161***</td>
<td>5818±182**</td>
<td>1391±29***</td>
</tr>
<tr>
<td>Cd+Se</td>
<td>6461±143</td>
<td>5251±122</td>
<td>1177±56</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 6 animals in each group. Significantly different from controls. **p<0.02 and ***p<0.01

Table 3 shows the results on the activities of total SOD, CuZn SOD and Mn SOD in the liver of rats after exposure to cadmium (Cd), selenium (Se), and cadmium + selenium (Cd + Se) compared with the controls (C). Total SOD, CuZn SOD and Mn SOD activities are presented in Table 3. In rats exposed to cadmium the activities of total SOD and Mn SOD were significantly decreased (p<0.02), while the activity of CuZn SOD was not significantly altered. In rats exposed to selenium the activities of total SOD
(p<0.02), CuZn SOD (p<0.02) and Mn SOD (p<0.01) were significantly enhanced. However, the concomitant exposure of rats to both cadmium and selenium did not change the activity of the enzymes studied.

Data on CAT, GSH-Px, and GST activities are presented in Table 4. In animals exposed to cadmium the activities of CAT and GSH-Px were significantly diminished (p<0.005), while the activity of GST was significantly higher (p<0.005) as compared to control animals. After exposure to selenium, the activities of CAT and GST (p<0.02) were significantly increased. The concomitant exposure to both cadmium and selenium did not affect the activities of CAT and GSH-Px, while the activity of GST was significantly increased (p<0.02).

Table 5 shows the data on ascorbic acid (AsA) and vitamin E (vit E) concentrations in the liver. Exposure to cadmium significantly decreased AsA (p<0.005) and concomitantly increased vitamin E concentration (p<0.005) in the liver of rats. In animals exposed to selenium, both the concentrations of AsA (p<0.02) and vitamin E (p<0.01) were augmented. Concomitant treatment with cadmium and selenium significantly decreased the concentration of AsA (p<0.01) and increased the concentration of vitamin E (p<0.05).

**Table 4**  
The activities of catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) in the liver of rats after exposure to cadmium (Cd), selenium (Se), and cadmium + selenium (Cd + Se) compared with the controls (C).

<table>
<thead>
<tr>
<th></th>
<th>CAT (U/g w.m.)</th>
<th>GSH-Px (U/g w.m.)</th>
<th>GST (U/g w.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>56 289±699</td>
<td>62 631±1388</td>
<td>76 872±3824</td>
</tr>
<tr>
<td>Cd</td>
<td>48 500±623****</td>
<td>47 661±717****</td>
<td>99 819±2434***</td>
</tr>
<tr>
<td>Se</td>
<td>61 598±2986**</td>
<td>65 385±1178</td>
<td>92 944±1824**</td>
</tr>
<tr>
<td>Cd+Se</td>
<td>52 700±4647</td>
<td>63 192±753</td>
<td>95 069±3080**</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 6 animals in each group. Significantly different from controls. **p<0.02 and ****p<0.005.

**Table 5**  
Ascorbic acid (AsA) and vitamin E (vit E) concentrations in the liver of rats after the exposure to cadmium (Cd), selenium (Se) and cadmium + selenium (Cd + Se) compared with the controls (C).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cd</th>
<th>Se</th>
<th>Cd+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsA (mg%)</td>
<td>37.6±0.84</td>
<td>30.0±0.39****</td>
<td>45.2±1.58**</td>
<td>31.5±0.50***</td>
</tr>
<tr>
<td>vit E (µg/g)</td>
<td>13.4±0.69</td>
<td>23.3±0.65****</td>
<td>26.9±1.50***</td>
<td>16.0±0.48*</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 6 animals in all groups. Significantly different from controls. *p<0.05; **p<0.02; ***p<0.01 and ****p<0.005.

**Discussion**

The results of former investigations have shown that cadmium decreases body growth in adult and young animals (Prigge et al. 1977, Rajanna et al. 1984, Štajn et al. 1992). The data obtained in this study are in accordance with the previous investigations and show that the body growth of rats exposed to cadmium is smaller by 65% in comparison to control animals (Table 1). At the same time, the mass of the liver was also decreased, as indicated by lower relative liver mass. Similar changes in the liver were observed in previous investigations (Schnell 1978, Sunderman 1978, Dudley et al. 1984) and they were considered to be a consequence of liver injury and diminished protein synthesis caused by the toxic influence of cadmium (Olsson 1986). Our results show that a multiple increase in the liver content of this metal occurs in rats...
exposed to cadmium, which is in accordance with other investigators (Probst et al. 1977, Wahba et al. 1993). It is interesting that with increasing concentrations of cadmium in the liver, the concentration of selenium also rises, although it was not administered additionally (Table 1). The increased concentration of selenium in the liver could be explained by its redistribution from other tissues and organs (Jamall and Smith 1985c) as well as by the formation of Cd-Se protein complexes (Magos and Webb 1980). In rats exposed to selenium, no significant changes of body mass and liver mass were observed, except of the increased accumulation of selenium. When rats were concomitantly exposed to both cadmium and selenium, decreased growth of body mass and liver mass was observed but these values were significantly increased in comparison with rats exposed to cadmium only (Table 1). By concomitant exposure of rats to both cadmium and selenium the accumulation of both elements increased in liver. All these data indicate that selenium diminished the toxic effects of cadmium on body and liver growth to a significant degree and increased the accumulation of cadmium in the liver (Wahba et al. 1993).

The data obtained in our study (Table 2) confirm that chronic intoxication with cadmium causes a significant decrease of haemoglobin concentration (Prigge et al. 1977, Kunimoto et al. 1986, Kostić et al. 1993) and an increase of blood glucose concentration in rats (Chapawala et al. 1980). Our data also show that after exposure to selenium these values remain at the control level. In rats concomitantly exposed to both cadmium and selenium, the toxic effects of cadmium on blood glucose were completely abolished, while the negative effects on haemoglobin concentration were significantly diminished.

Previous investigations have shown that cadmium inhibits the activity of most enzymes involved in the antioxidant defense system (Jamall and Smith 1985c, Shukla et al. 1987) and increases the production of free radicals, lipid peroxidation and destruction of cell membranes (Amoruso et al. 1982, Chung and Maines 1987, Hussain et al. 1987, Kumar et al. 1992). Our results confirm the previous findings and show that cadmium significantly inhibits the activities of total SOD, Mn SOD, and CAT and GSH-Px (Tables 3 and 4). The decreased activity of GSH-Px can be explained by competition of Cd-metallothioneins and GSH-Px for sulphur containing amino acids (Olsson 1986). At the same time the activity of enzyme GST was significantly enhanced (Table 4). This enzyme is important in detoxication of lipid peroxides and for protection of integrity and functioning of cells and tissues. It is characteristic that, in the liver of rats exposed to selenium, the activities of all enzymes of the antioxidant defense system were increased, including the activity of enzyme GST. By concomitant exposure of rats to both cadmium and selenium, the activities of total SOD, CuZn SOD, Mn SOD, CAT and GSH-Px remain at the level of control values, indicating that selenium eliminates the toxic effects of cadmium on the activity of these enzymes. However, the activity of GST in the liver of these animals was increased. This can be explained, in rats exposed to cadmium and selenium separately, by an important role played by this enzyme in preventing lipid peroxidation and oxidative damage of the liver (Chakrabarty et al. 1992).

Our data (Table 5) show that cadmium significantly decreases ascorbic acid concentration in the rat liver, which is in accordance with the results of other investigators (Chatterjee et al. 1973, Shukla and Chandra 1989). It is known that increased accumulation of cadmium in the liver induces lipid peroxidation and increases the production of malondialdehyde (MDA), which consequently inhibits the enzyme L-gulonolactone oxidase (Chatterjee et al. 1960, Hudecová and Ginter 1992) necessary for the synthesis of AsA. Ascorbic acid is a potent scavenger of superoxide anion radicals and singlet oxygen and it has been shown that marginal AsA deficiency results in intracellular oxidative damage in the guinea-pig (Chakrabarty et al. 1992, Kadrabová et al. 1992, 1993, Mozesova and Ginter 1992, Nagyová et al. 1884, Tatara and Ginter 1994) and the hamster (Nagyová et al. 1993).

In rats exposed to increased concentrations of selenium, ane opposite effect was obtained (Table 5), so that the concentration of AsA in the liver was significantly increased. From our results it can be concluded that the inhibitory effect of cadmium on the concentration of AsA is more marked than the stimulatory effects of selenium, since the concentration of AsA in the liver was significantly lower in rats concomitantly exposed to both cadmium and selenium than in control animals.

Both cadmium and selenium increase the concentration of vitamin E in the rat liver significantly (Table 5). This vitamin is an important antioxidant which acts as a scavenger of free radicals, inhibits lipoxygenases and reduces peroxides in association with lipoxygenases (Burton et al. 1989, Shukla and Chandra 1989, Kumar et al. 1992). Our results show that both cadmium and selenium in the given concentrations induce oxidant stress, and that vitamin E, in addition to enzyme GST, plays an important role in its prevention. In rats concomitantly exposed to both cadmium and selenium, the concentrations of vitamin E were significantly decreased as compared to rats exposed to cadmium or selenium separately, and reaches control values. These data indicate that the toxic influence of cadmium is possibly decreased.

In conclusion, cadmium in the liver inhibits the activity of multiple enzymes of the antioxidant defense system (SOD, CAT, GSH-Px), except for GST the activity of which is increased. The enhanced activity of GST, and the increased concentration of vitamin E as a nonenzymatic component of the antioxidant defense system may be due to a defense response of the
organism to oxidant injuries caused by cadmium, since the activities of other enzymes as well as the concentration of AsA in the liver were decreased. By exposure of rats to higher than physiological concentrations of selenium, the activities of all enzymes of the antioxidant defense system are augmented. The same is true for the concentrations of AsA and vitamin E. The concomitant exposure of rats to both cadmium and selenium eliminated the toxic effects of cadmium completely (SOD, CAT, GSH-Px) or diminished them (AsA). However, the activity of GST as well as the concentration of vitamin E significantly rose in comparison to control values. It can thus be concluded that in the liver of these animals there is a "need" for the prevention of damage caused by oxidant stress. According to the results of previous studies, the reduced toxicity of cadmium can be explained by the formation of Cd-Se protein complexes (Magos and Webb 1980), as well as by redistribution of cadmium from proteins of low molecular mass into the proteins of high molecular mass (Gasiewicz and Smith 1976, Viljoen and Tappel 1988).

Acknowledgements
This research was supported by the National Ministry of Science, Technology and Development, Grant 2/0-4 No. TSI-380/94-2.

References


**Reprint Requests**

Prof. Dr. V.M. Petrović, Member of Serbian Academy of Sciences and Arts, Department of Physiology, Institute for Biological Research "Siniša Stankovic", 29. November 142, 11060 Belgrade, Serbia, Yugoslavia.