DOI: 10.2429/proc.2012.6(1)009 2012;6(1)

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# INFLUENCE OF COPPER ON SUPEROXIDASE DYSMUTASE ACTIVITIES IN RATS EXPOSED TO CADMIUM

## WPŁYW MIEDZI NA AKTYWNOŚĆ DYSMUTAZY PONADTLENKOWEJ U SZCZURÓW ZATRUWANYCH KADMEM

**Abstract:** The aim of this study was to evaluate the influence of copper supplements on superoxide dismutase (SOD) activities in the kidneys and liver of rats exposed to moderate doses of cadmium chloride. SOD activities were measured weekly for a period of 28 days. The examinations involved male Wistar rats divided into three groups. Rats in group I were considered the controls whereas rats in group II and III were administered intragastrically for 7 d, 14 d, 21 d, and 28 d a water solution of cadmium chloride corresponding to 10 mg Cd/kg diet. All rats fed a standard laboratory chow LSM (Agros Motycz, Poland) containing 5 mg Cu/kg except for the rats in group III in which animals were fed an LSM diet supplemented up to 50 mg Cu/kg. The experimental procedure included evaluation of water and feed intake, body weight gains, liver, kidneys, testicles, heart and spleen to body ratio, and hepatic and renal SOD activities measured after 7 d, 14 d, 21 d, and 28 d exposure to cadmium or cadmium plus copper. Results indicated that SOD activity in cadmium or cadmium plus copper groups changed in comparison with the control values. Supplements of copper increased significantly hepatic and renal SOD activities after 21 and 28 d of exposure when compared to those found in the controls and cadmium exposed rats. Moreover, rats fed the copper supplement diet improved body weight gain in comparison with that in the cadmium exposed rats. Results suggest that copper may stimulate a protective activity against toxic action of cadmium given at doses resembling its concentrations in the areas contaminated with this metal.

**Keywords:** copper, cadmium, superoxide dismutase, rat

Cadmium is constantly released into the atmosphere with an estimated 4 000 to 13 000 Mg (tons) coming from human activities. The cadmium present in the environment does not break down. Consequently, plant, animal and human exposure to and intoxication with cadmium continues to occur [1]. Following exposure, cadmium is readily distributed to all organs and tissues where it proceeds toxic activities [2]. Its participation in biological functions may lead to oxidative stress causing the loss of membrane integrities in biomolecules including lipids and proteins [3].

Animal studies have provided evidence that several factors may affect the absorption and toxicity of cadmium [4-6]. For example, Fox [7] discussed the effects of dietary nutrients in increasing or decreasing risks from cadmium toxicity. It was found that a diet deficient in copper and iron caused increased mortality and reduced growth in chicks whereas copper supplements decreased mortality and increased haematocrit of anaemic Japanese quail fed cadmium in the diet. Moreover, it was reported that rats fed a copper fortified diet (10 times the level in a standard diet) increased cadmium content in metabolically significant organs including liver and kidneys [8]. However, the increases in cadmium content were associated with improved body gains suggesting that the toxic action within the body of rats exposed to cadmium was limited.

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It worth noting that relationships between cadmium and copper seems to be significant not only during metabolism but also when enzyme activities are considered [6].

The aim of these studies was to evaluate the effect of copper supplements on the renal and hepatic activity of *superoxide dismutase* (SOD) in rats exposed to dietary doses of cadmium. The studies included SOD activities because this enzyme constitutes one of the main defense mechanisms against the oxidative stress produced by cadmium.

## Material and methods

Experiments involved male Wistar weighing 207 ± 12 g. After a one-week acclimatization period rats were randomly assigned to three groups (45 animals per group). Animals in group I (control) were fed a standard laboratory diet LSM (Fodder Manufacture Motycz, Poland) and drunk tap water whereas rat in groups II and III were exposed daily for 28 days (except weekends) to a water solution of cadmium chloride containing cadmium at a concentration corresponding to 10 mg Cd/kg of diet. Cadmium chloride was administered intragastrically using a stainless gavage. Rats in groups II were fed the LSM diet containing 5 mg Cu/kg (according to the manufacture's procedure) whereas rats in group III the LSM diet supplemented with copper chloride up to 50 mg Cu/kg. The experimental procedure included evaluation of water and feed intake, body weight gains, organ to body ratios, and renal and hepatic SOD activities measured according to Marklund's method [9] after 7 d, 14 d, 21 d, and 28 d intoxication with cadmium. Rats were killed by immersion in gaseous carbon dioxide.

Statistical analysis of results based on 5 rats killed at each point time. Mean values were compared by Student's *t*-test at $P < 0.05$.

## Results

Rats in all groups demonstrated a similar feed and water intake. The body weight gains measured after 28-day intoxication with cadmium were visibly lower in rats fed a standard diet and exposed to cadmium as compared with those in the controls and group III. However, the differences were not statistically significant (Table 1). Further, the organ to body ratio for the liver, spleen, heart, and kidneys was similar in the three groups examined except for the testicles showing a statistically significant decrease in group III in comparison with the value in the controls.

Table 1

Body weight gains and organ to body ratio measured after a 28-day intoxication

| Groups | Initial weight [g] | Final weight [g] after 28 d exposure | Liver to body ratio | Kidneys to body ratio | Testes to body ratio | Heart to body ratio |
|---|---|---|---|---|---|---|
| Control | 190 ± 12 | 348 ± 45 (87%) | 3.36 ± 0.32 | 0.71 ± 0.06 | 1.13± 0.14 | 0.23 ± 0.03 |
| Group II | 202 ± 12 | 325 ± 21 (61%) | 3.76 ± 0.37 | 0.73 ± 0.06 | 0.85 ± 0.09 | 0.26 ± 0.02 |
| Group III | 208 ± 12 | 366 ± 44 (78%) | 3.92 ± 0.38 | 0.68 ± 0.06 | 0.79 ± 0.11* | 0.27 ± 0.03 |

* means statistically significant differences at $P < 0.05$

The effect of increasing doses of cadmium on superoxide dismutase activities in the liver and kidneys were shown in Tables 2 and 3. A gradual increase in the renal and hepatic

activities of SOD was observed in the controls, cadmium, and cadmium plus copper treated rats. Maximum values were noted after 21 days in the controls and cadmium treated rats whereas in the rats exposed to cadmium and supplemental copper peak values were seen at 28 days. It should be also stressed that SOD activities in groups II and III were a little lower after 7 days intoxication with cadmium. No significant differences in SOD activities were found between groups I and II. However, rats exposed both to cadmium and supplemental copper demonstrated significant increases in renal and hepatic SOD activities after 21 days in comparison with those in the controls and significant increases after 28 days as compared to those found in the controls and cadmium exposed group.

Table 2

Hepatic SOD activities in rats exposed to cadmium and copper

| Intoxication [days] | Control (Group I) | Cadmium (Group II) | Cadmium and copper (Group III) |
|---|---|---|---|
| 7 | 48.26 ± 14.56 | 40.37 ± 13.76 | 45.88 ± 13.56 |
| 14 | 53.57 ± 18.12 | 56.96 ± 20.09 | 100.61 ± 23.81 |
| 21 | 77.33 ± 17.89 | 85.27 ± 22.43 | 134.93 ± 31.50[a] |
| 28 | 56.54 ± 14.32 | 69.49 ± 17.67 | 140.09 ± 29.69[ab] |

Explanations: a - means statistically significant differences between group III and groups I at $P < 0.05$, b - means statistically significant differences between group III and groups II at $P < 0.05$

Table 3

Renal SOD activities in rats exposed to cadmium and copper

| Intoxication [days] | Control (group I) | Cadmium (group II) | Cadmium and copper (group III) |
|---|---|---|---|
| 7 | 38.77 ± 12.93 | 31.56 ± 12.74 | 35.08 ± 11.85 |
| 14 | 39.63 ± 15.08 | 51.16 ± 16.07 | 72.02 ± 16.12 |
| 21 | 56.15 ± 17.29 | 68.62 ± 16.94 | 127.69 ± 25.84[a] |
| 28 | 47.46 ± 17.02 | 62.66 ± 15.67 | 136.96 ± 27.06[ab] |

Explanations as in Table 2

## Discussion

The cadmium doses given to rats in this experiment corresponded to 10 mg Cd/kg diet. The toxicological consequence of cadmium given at such dose for relatively short time is not well known. Examinations of the kidneys, testicles and liver of rats exposed to similar amounts of cadmium failed to show any anatomopathological alterations in these organs (not yet published). Several authors reported no responses in growth, feed and water consumption in ewes, lambs and pigs given cadmium at concentrations up to 30 mg/kg [10, 11]. However, it should be stressed that in the present studies the relative weight of testicles decreased significantly in rats exposed both to cadmium and copper. The toxicity of cadmium to the male reproductive system has been extensively investigated in animals. It was reported that long-term treatment with moderate doses of cadmium for several months did not give rise to any toxicity to testicles [12]. A drop in relative testicular weight in the case of concomitant supplemental copper administration may correspond to reports by Chattopadhyay et al [15] who found significant reduction of testicular and accessory sex organs (seminal vesicle, ventral prostate) weight after intraperitoneal injection of copper chloride at doses of 2 and 3 mg/kg body weight/day for 26 days.

Cadmium may cause indirectly prolonged generation of *reacitive oxygen species* (ROS) which promote necrosis. Superoxide dismutase is an essential cellular component of antioxidative defense system as it dismutates two oxygen radicals to oxygen and water.

In the present studies the activities of SOD in the liver and kidneys of rats exposed cadmium and cadmium plus copper decreased after 7 day exposure. A drop in SOD activities on day 7 may reflect an enhancement in lipids peroxidation with concomitant impairment in the antioxidative defence mechanism [14]. However, continuous exposure to cadmium and copper for a longer time stimulated a steady-state increase in SOD activities on days 14, 21, 28. These increases in SOD activities seems to be attributed to the supplements of copper and confirmed findings of others who also found enhanced SOD activities in patients fed a diet fortified with this element [16].

On the basis of the results presented here, it can be concluded that copper supplements given to rats exposed to a moderate level of dietary cadmium improve the body weight gain within the exposure period. Moreover, an increase in SOD activities may indicate an increased protection from oxidative stressed produced by cadmium.

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**Abstrakt:** Celem pracy było określenie wpływu diety wzbogaconej w miedź na aktywność *dysmutazy ponadtlenkowej* (SOD) w wątrobie i nerkach szczurów szczepu Wistar eksponowanych na umiarkowane dawki chlorku kadmu. W badaniach uwzględniono 3 grupy zwierząt: grupę I stanowiły zwierzęta kontrolne, grupę II zwierzęta eksponowane na kadm podawany dożołądkowo w ilościach odpowiadających 10 mg Cd/ kg paszy oraz grupę III, w której kadm podawano tak jak w grupie II, a ponadto zwierzęta eksponowane były na chlorek miedzi podawany w paszy LSM (Agros Motycz, Polska) w stężeniu 50 mg Cu/kg. Stężenie miedzi w standardowej paszy LSM podawanej zwierzętom w grupach I i II wynosiło 5 mg Cu/kg. W doświadczeniu określano spożycie paszy i wody, przyrosty masy ciała, masy względne wątroby, nerek, serca, śledziony i jąder oraz aktywność SOD po 7, 14, 21 i 28 d podawania kadmu. Wyniki badań wskazują, że zatruwanie szczurów chlorkiem kadmu zmieniało aktywność SOD w wątrobie i nerkach w porównaniu z danymi w grupie kontrolnej. Dodatek miedzi do paszy zwiększył statystycznie istotnie aktywność SOD po 21 i 28 dniach ekspozycji na kadm w porównaniu z danymi w grupie kontrolnej i grupie eksponowanej tylko na kadm. Warto także podkreślić, że dodatek miedzi do paszy szczurów zatruwanych kadmem powodował zwiększone przyrosty masy ciała w porównaniu z danymi uzyskanymi od zwierząt karmionych standardową paszą i eksponowanych na kadm. Uzyskane wyniki sugerują, że miedź może mieć ochronne działanie u zwierząt eksponowanych na kadm drogą pokarmową w ilościach, które mogą być obecne w paszy na terenach skażonych tym metalem.

**Słowa kluczowe:** miedź, kadm, dysmutaza ponadtlenkowa, szczur