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Cadmium induced lipid peroxidation in rat testes and protection by selenium

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Abstract

The main goal of this study was to investigate the role of cadmium in the promotion of lipid peroxidation in the homogenates of rat testes and the effect of selenium on lipid peroxidation in testes of rats after cadmium injection. Treatment of rats with cadmium resulted in a time- and dose-related accumulation of the metal ions in testes. The concentrations of cadmium, copper, zinc, selenium and iron in the tissues were determined by an atomic absorption spectrophotometer and lipid peroxidation in testes was measured by a spectrophotometer. Cadmium produced enhanced lipid peroxidation in testes. These cadmium-induced changes were accompanied by a significant increase of iron and copper, and a decrease of zinc in testes. Concurrent treatment with selenium and cadmium reduced the cadmium-induced alterations in lipid peroxidation and essential metal levels. Data suggest that lipid peroxidation was associated with cadmium toxicity in testes and that the addition of selenium was found to be effective in attenuation of this effect.

Introduction

Cadmium (Cd) is perhaps one of the most toxic industrial and environmental metals, and it poses a continuing health hazard. The reproductive toxicity of Cd as an industrial pollutant, a food contaminant, and contribution from cigarette smoke is well established. (Wahba *et al.* 1993; Kadrabova *et al.* 1993). Cd can cause a number of lesions in many organs, such as the liver, kidney and testes. The prominent effects of Cd on testicular tissue are well recognized, as early as 1919, Alsberg and Schwartze (1919) had noted that administration of Cd salts in animals caused 'bluish discoloration of the testicles'. The testes seem to be extremely sensitive to Cd as hemorrhagic necrosis of rodent testes can be induced by Cd concentrations as low as 0.15μg/g of body weight (Nolan & Shaikh 1986), and Cd induces severe necrosis followed by chronic degeneration in the rodent testes (Waalkes *et al.* 1997) . Exposure to low levels of cadmium reduces fertility, because in male mice spermatogenesis is highly sensitive to Cd (Dalton *et al.* 1996).

The role of lipid peroxidation in living tissues has received considerable attention as a potential health hazard of exposure to certain metals. Lipid peroxidation, which is an exceedingly damaging process, has been known to occur via peroxidation of unsaturated fatty acids in all aerobic biological system. Free radical damage to phospholipids is an important factor in the development of toxic conditions. The ions of certain inorganic compounds, such as iron and copper, are powerful promoters of free radicals. Also, other ions like cadmium, cobalt, mercury, nickel, tin, lead and vanadium are known to generate lipid peroxidation in target tissues of rodents (Sunderman 1986; Manca *et al.* 1991; Shafiq-ur-Rehman *et al.* 1995). In agreement with these observations, several studies (Fariss 1991; Reitier *et al.* 1998; Sarkar *et al.* 1998; Shaikh

et al. 1999; Yiin *et al.* 1999) indicated that free radical scavengers and antioxidants are useful in protecting against cadmium toxicity.

In the present study, we were interested in determining the interrelationship of essential nutrients such as Fe, Cu, Zn, Se and Cd in metal-associated toxicity, and an attempt has been made to examine the role of cadmium in the promotion of lipid peroxidation in the homogenates of rat testes. The protective effects of selenium against the testicular toxicity caused by acute exposure to cadmium was also studied.

Materials and methods

Animals and treatments

Male Sprague-Dawley rats, weighing 330–420 g, were purchased from the National Laboratory Animal Breeding and Research Center of the Republic of China, and were kept in stainless-steel mesh cages, housed under controlled conditions(22 ± 2 °C, $50 \pm$ 20% relative humidity, 12 h light-dark cycle) with rat diet and drinking water ad libitum. Five rats were usually tested per group in each experiment. For the dose-effect study, rats were injected intraperitoneally with $CdCl_2$ at doses of 25, 125, 500 or 1250 μg Cd/kg. The controls were administered saline (0.9 ml/kg), and decapitated 24 h after the injection. For the time-course experiment, rats were injected intraperitoneally with $CdCl_2$ (25 or 500 μg Cd/kg), and killed at 6, 12, 24 or 72 hours after metal injection. For the study of effects of antioxidants, rats were injected intraperitoneally with $CdCl_2$ (500 μg Cd/kg) and antioxidant, sodium selenate (Na_2SeO_4, 125 μg Se/kg). These rats were killed at 6, 12, 24 or 72 hours after sodium selenate treatment. The testes were immediately excised, blotted, and then rinsed with cold 0.9% NaCl solution.

Thiobarbituric acid reactive substances (TBARS) assay

The presence of this TBARS is an index of lipid peroxidation (Kitabchi & Williams 1968). Testes (1 g, wet wt) was homogenized in 10 ml cold KCl solution using motor-driven tissue homogenizers with Teflon pestles. Lipid peroxidation was determined by the thiobarbituric acid assay according to Sunderman *et al.* (1985). Duplicate aliquots of each homogenate (0.2 ml) were pipeted into Pyrex tubes (13 mm diameterm, 150 mm length) containing 3 ml cold 1% (v/v) H_3PO_4 solution. Standard solutions were prepared by pipeting 0.2 ml of each tetraethoxypropane (TEP) working standard solution (0, 3. 3, 6. 6, 9. 9, 13.2 nmol/0.2 ml) into Pyrex tubes containing 3 ml cold H_3PO_4 solution. A 0.2-ml ethanol solution was used as blank. A cold KCl solution was added to each tube to dilute the contents to 4 ml. One milliliter of TBA solution was then added to each tube, and the contents mixed with a vortex mixer. After mixing, the tubes were capped with marbles and placed in boiling water for 45 min. The tubes were then cooled to room temperature in a basin containing tap water. After cooling, 4 ml butanol were added to each tube, and the contents mixed for 20 s with a vortex mixer. The contents of each tube were transferred to a conical polystyrene centrifuge tube (12 ml vol) , capped, and centrifuged for 20 min (1000 g, 25 °C). Each butanol extract was transferred with a Pasteur pipet into a spectrophotometer cuvet (1 cm light path). Spectrophotometric scan was performed from 500–560 nm, using a spectrophotometer, with butanol in the reference cuvet. The TBA chromogens were determined at maximum absorption (532 nm).

Determination of metal concentrations

The testes were wet-digested following the perchloric acid-nitric acid method (Kanno *et al.* 1994). Cadmium and the essential metals, Fe, Cu, Zn, Se, were determined using a Perkin-Elmer model 5100 PC atomic absorption spectrophotometer as described in our previous study (Jang *et al.* 1996). The detection limit and precision of the metal analysis was shown in Table 1.

Data analysis

Statistical analysis was performed with SAS software. The statistical analysis of data was performed by wilcoxon two-sample test and a p-value less than 0.05 was considered to be statistically significantly.

Results

Cadmium accumulation in testes

Table 2 listed the amounts of cadmium in testes of rats 24 h after administration of various doses of $CdCl_2$. The testes Cd concentration accumulation was dose-dependent. The Cd in testes of rats injected with $CdCl_2$ 25 or 500 μg/kg at 6, 12, 24 or 72 h is shown in Table 3. Testes Cd accumulation increased with time and

Table 1. The detection limit and precision of Cd, Fe, Cu, Zn and Se

	Cd	Fe	Cu	Zn	Se
Detection limit	0.08 ppb	0.33 ppb	0.36 ppb	0.008 ppm	0.1 ppb
precision (CV%)[a] $N = 5$					
Within-run	2.6%	3.4%	1.1%	0.6%	3.9%
Between-run	6.7%	4.8%	3.5%	1.5%	3.9%

[a]CV: coefficient of variance.

Table 2. Essential metal concentrations in testes of rats 24 hours after administration of various doses of cadmium chloride[a]

Cd dose		Metal (μg/g wet tissue)			
μg/kg	Cd	Fe	Cu	Zn	Se
Control	ND[b]	17.7 ± 0.9	3.04 ± 0.4	27.5 ± 2.5	0.736 ± 0.084
25	0.129 ± 0.094[c]	23.8 ± 5.0	4.45 ± 1.5	27.7 ± 0.7	0.873 ± 0.275
125	0.490 ± 0.047[c]	34.9 ± 2.8[c]	5.17 ± 1.2[c]	26.0 ± 0.6	0.880 ± 0.147
500	0.675 ± 0.287[c]	38.0 ± 3.0[c]	7.4 ± 1.7[c]	25.7 ± 1.6	0.670 ± 0.123
1250	1.147 ± 0.592[c]	45.9 ± 9.4[c]	11.8 ± 2.1[c]	22.4 ± 1.3[c]	0.603 ± 0.067

[a]Data are the mean $\pm$ s.e. of 5 rats per group.
[b]ND is not detected.
[c]Significantly different from control ($p < 0.05$).

was dose-related. The highest concentrations found in this study occurred at 72 h with both doses.

Lipid peroxidation in testes

Data showing the dose-response effect of Cd on lipid peroxidation in the testes of rats are given in Table 4. There was a significant increase in lipid peroxidation products in testes following treatment with all doses of metal used beside the 25 μg/kg Cd. But a significant rise was noted at 72 h with the 25 μg dose. The 500 μg/kg Cd dose markedly altered testes lipid peroxidation at 24 and 72 h (Table 5).

Cd effects on testes essential metal concentrations

Data in Table 2 show that Cd at 125, 500 and 1250 μg/kg significantly increased testes Fe and Cu levels. In contrast, a significant decrease was noted in testes Zn after administered with 1250 μg/kg Cd. In Table 3, data listed that significant increase was shown in testes Fe and Cu at 24 and 72 h after treated with 500 μg/kg Cd, however, testes Zn significant decrease occurred at 72 h after administered with 500 μg/kg Cd.

Effects of Se on Cd-induced changes in testes

Data in Table 6 show that Se at 24 and 72 h significantly lowered lipid peroxidation in testes. This was accompanied by a significant fall in testes Cd and testes Cu at 24 and 72 h and a significant decrease in testes Fe at 6, 12, 24 and 72 h, however, a significant increase in testes Zn after $CdCl_2$ combined with Na_2SeO_4 injection. As expected the concentration of Se in testes was markedly higher in the rats receiving Se plus Cd compared to the Cd alone group.

Discussion

Cadmium is one of many metals that are not physiologically or biochemically essential to organisms. Cd distrubutes to tissues rapidly and has a high volume of distribution (Waalkes *et al.* 1992). The administration of cadmium during organogenesis causes growth retardation, congenital malformations, and fetal death in rodents. Acute cadmium exposure induces vascular destruction in the testes (Sugawara *et al.* 1989; Chubb 1992). Cadmium-induced hypoxia/ischemia produces a series of secondary effects, including seminiferous tubule necrosis and Leydig cell damage (Itoh 1985; Damber *et al.* 1987), despite numerous studies, the primary action of cadmium remains unknown. This

Table 3. Time-course effects of Cd on essential testes metal concentrations[a]

Dose (μg/kg)	Time (h)	Metal (μg/g wet tissue) Cd	Fe	Cu	Zn	Se
25	0	ND	17.7 ± 0.9	3.0 ± 0.4	27.5 ± 2.5	0.736 ± 0.084
	6	0.071 ± 0.012[c]	24.6 ± 2.5	2.4 ± 0.7	27.6 ± 2.1	0.890 ± 0.172
	12	0.124 ± 0.029[c]	25.0 ± 2.1	3.1 ± 0.9	27.4 ± 1.9	0.875 ± 0.164
	24	0.339 ± 0.072[c]	33.8 ± 5.0[c]	4.5 ± 1.5	27.7 ± 0.7	0.873 ± 0.275
	72	0.129 ± 0.072[c]	37.8 ± 2.5[c]	4.7 ± 1.9	25.6 ± 0.9	0.808 ± 0.158
500	0	ND	17.7 ± 0.9	3.0 ± 0.4	27.5 ± 2.5	0.736 ± 0.084
	6	0.473 ± 0.337[c]	25.6 ± 2.1[c]	3.7 ± 1.2	27.1 ± 1.4	0.749 ± 0.091
	12	0.516 ± 0.042[c]	27.0 ± 2.8[c]	5.1 ± 1.9	26.9 ± 0.8	0.721 ± 0.102
	24	0.675 ± 0.287[c]	38.0 ± 3.0[c]	7.4 ± 1.7[c]	25.7 ± 1.6	0.670 ± 0.123
	72	0.962 ± 0.280[c]	40.1 ± 4.2[c]	9.2 ± 2.1[c]	23.6 ± 1.1[c]	0.603 ± 0.067[c]

[a]Data are mean $\pm$ s.e of 5 rats per group.
[b]ND is not detected.
[c]Significantly different from respective control ($p < 0.05$).

element is extremely dangerous as it is easily absorbed and remains in tissues for a long time. Exposure to high doses of cadmium may cause biochemical and functional changes in some critical organs. Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids and altering the antioxidant system of the cells (Sarkar *et al.* 1995). The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with cellular organelles. In the testes, the effects of cadmium are more important in the reduction of seminiferous tubule diameter (Corpas & Antonio 1998), and enhanced lipid peroxidation may provide a basis for cadmium induced testes toxicity. Swiergosz *et al.* (1998) reported that histological examination of the tissue revealed some pathological changes in the structure of testes, and subcutaneous injections of cadmium salts in animals result in capillary stasis, edema, and hemorrhages in the testis (Parizek 1956, 1957). These events are followed by regressive changes of the seminiferous epithelium 4–6 h after injection and total necrosis within 24–48 h. In the present study, cadmium accumulation in the testes was directly proportional to dose and accumulation of testes Cd was accompanied by increased testes Cu and Fe concentrations in a dose-related fashion. Cu and Fe may act as catalyst in the Fenton/Haber–Weiss reactions, facilitating the conversion of superoxide anion, and hydrogen peroxide to hydroxyl radical species, which are believed to initiate lipid peroxidation (Kjellstrom & Nordberg 1978). Furono *et al.* (1996) suggested that the addition of

Table 4. Effect of various Cd doses on testes lipid peroxidation[a]

Cd dose (μg/kg)	Lipid peroxidation products (μmol TBA-chromogens/g wet tissue)
Control	64.8 ± 19.8
25	110.3 ± 12.5
125	124.8 ± 12.1[b]
500	129.4 ± 18.5[b]
1250	140.5 ± 19[b]

[a]Data are the mean$\pm$s.e. of 5 rats per group.
[b]Significantly different from control ($p < 0.05$).

Cu caused substantial lipid peroxidation in normal hepatocytes. And Koizumi *et al.* (Koizumi & Li 1992) showed that lipid peroxidation levels and Fe content were remarkably elevated in testicular after treatment of rats with a single dose of $CdCl_2$ (30 μmol/kg). Zn might be essential for normal metabolic activity and /or tissue ingetrity at some testicular sites, where Cd could be displacing the Zn (Arvidson 1983). In the present study it is shown that Zn decreased significantly after administering 1250 μg Cd/kg, and 500 μg Cd/kg, 72 h. Several authors (Singhal & Merali 1979; Maitani & Suzuki 1986; Sugawara *et al.* 1989) found that Cd administration can displace significant amounts of testicular Zn. Since Zn is essential for the maintenance of germinal epithelium, it has been suspected that Cd might exert its initial injurious effects on Zn-dependent spermatogenic elements.

Wahba *et al.* reported that selenium prevents the toxicity of the cadmium through undefined mecha-

Table 5. Time-course of lipid peroxidation in testes of Cd-treated rats[a]

Dose (μg/kg)	Time (h)	Lipid peroxidation products (μmol TBA-chromogens/g wet tissue)
25	Control	64.8 ± 19.8
	6	78.7 ± 14.8
	12	96.2 ± 18.3
	24	110.3 ± 12.5
	72	118.1 ± 16.2[b]
500	Control	64.8 ± 19.8
	6	82.3 ± 8.2
	12	114.7 ± 19.5
	24	129.4 ± 18.5[b]
	72	135.6 ± 17.6[b]

[a]Data are mean $\pm$ s.e. of 5 rats per group.
[b]Significantly different from respective control ($p < 0.05$).

Table 6. Effects of Se on Cd-induced alteration in testes[a]

Parameter	Time (h)	Cd alone	Cd □ Se
Testes TBA	6	105.3 ± 8.2	82.3 ± 48.5
(μmol-TBA-chromogens/g wet tissue)	12	104.7 ± 19.5	69.9 ± 20.0
	24	129.4 ± 18.5	50.1 ± 13.2[b]
	72	135.6 ± 17.6	38.1 ± 26.1[b]
Testes Cd	6	0.473 ± 0.337	0.409 ± 0.142
(μg/g wet tissue)	12	0.516 ± 0.042	0.489 ± 0.375
	24	0.675 ± 0.287	0.519 ± 0.036[b]
	72	0.962 ± 0.280	0.409 ± 0.142
Testes Fe	6	25.6 ± 2.1	15.7 ± 4.1[b]
(μg/g wet tissue)	12	27.0 ± 2.8	14.6 ± 1.6[b]
	24	38.0 ± 3.0	18.9 ± 5.3[b]
	72	40.1 ± 4.2	15.7 ± 4.1[b]
Testes Cu	6	3.7 ± 1.2	3.6 ± 0.4
(μg/g wet tissue)	12	5.1 ± 1.9	3.1 ± 0.7
	24	7.4 ± 1.7	2.9 ± 0.4[b]
	72	9.2 ± 2.1	3.6 ± 0.4
Testes Zn	6	27.1 ± 1.4	27.3 ± 2.9
(μg/g wet tissue)	12	26.9 ± 0.8	28.1 ± 0.9
	24	25.7 ± 1.6	28.8 ± 3.3
	72	23.6 ± 1.1	27.3 ± 2.9
Testes Se	6	0.749 ± 0.091	0.745 ± 0.147
(μg/g wet tissue)	12	0.721 ± 0.102	0.757 ± 0.025
	24	0.670 ± 0.123	0.780 ± 0.120[b]
	72	0.603 ± 0.067	0.745 ± 0.147

[a]Data are mean $\pm$ s.e. of 5 rats per group, Cd was administered i.p at 500 μg/kg for 6, 12, 24 or 72 h. In the concurrent study Cd and Se were administered at 500 μg/kg and 125 μg/kg, respectively.
[b]Significance is $p < 0.05$ (Cd alone v.s Cd+Se). Statistic method is wilcoxon two-sample test.

nisms (Wahba *et al.* 1993). In this study, concurrent treatment with Se and Cd reduced Cd-induced testes lipid peroxidation after treatment 24 h, suggesting that the toxic effect of cadmium on the testes is decreased by selenium. Kadrabova *et al.* 1993) showed that high doses of antioxidant, vitamin C, in cadmium-treated guinea-pigs decreased the levels of copper in the testes. Rana & Verma (1996) also reported that Cd failed to induce lipid peroxidation in the liver and kidney in presence of Se. It has been suggested that the interaction between Se and Cd is mediated by endogenous glutathione, which reduces selenite to selenide compound (Iwata *et al.* 1981). The high lipoaffinity of this compound might alter (reduce) its distribution and thus toxicity in critical tissues (Masukawa *et al.* 1982). It is expected that glutathione peroxidase activity would be stimulated in animals fed supplementary selenium (Reiter & Wendel 1985). Shukla *et al.* (1989) reported that Cd lowers glutathione peroxidase activity. Hence the protective mechanisms found with Se may involve antioxidant enzymes generating glutathione. Rana and Verma (1996) demonstrated that Se protection is affected by changes in Cd metabolism and complex responses of glutathione dependent enzymes. In our previous study selenium was also found to provide protection against Cd-induced lipid peroxidation (Yiin & Lin 1998; Yiin *et al.* 1999). In this study showed that testes Se is decreasing in 'Cd alone' for 24 and 72 hours than 'Cd + Se' group, the reason might be that cadmium will decrease the bioavailability of selenium (Preston 1991) and inhibited the endogenous selenium level. On the other hand, a metal-binding protein, metallothionein (MT) having a high affinity for cadmium, has also been implicated to play a protective role against Cd-induced testicular necrosis. But Wahba *et al.* (1993) reported that selenium prevents acute cadmium toxicity through a mechanism that does not involve induction of metallothionein. Therefore, the mechanism of Se treatment may be due to the depression of nonprotein sulfhydryl groups in rat tissues. This suggests that selenium protects cells from toxic effects of cadmium by maintaining the availability of antioxidant nonprotein sulfhydryl groups, because cadmium affinity to sulfhydryl groups may reduce free cadmium level in testes, thus attenuating the toxic effect of Cd.

In conclusion, our results showed that the administration of cadmium increased the products of lipid peroxidation in testes. Selenium might be very useful in protection against Cd-induced lipid peroxidation, as the presence of Se reduced the Cd effects.

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