Short Communication

CHANGES OF EIGHT METALS IN
MOUSE TESTIS TREATED WITH
CADIUM AND/OR SELENIUMカドミウムおよびセレンウム同時投与
マウスの精巣での金属元素の変動

Key words: cadmium; selenium; calcium; magnesium; potassium; sodium; zinc; copper; iron; testis

In general, the cadmium (Cd) level in the testis is much lower than that in the kidney and liver in experimental animals exposed parenterally to Cd.¹⁾ However, Cd brings about a very rapid progression of changes in the testis, including irreversible depletion of testicular function accompanied by biochemical,²⁾ histological³⁾ or physiological⁴⁾ changes. Of these, the reduction in Zn level has been well demonstrated⁵⁾ in relation to the important role of Zn in the spermatogenic epithelium and to the similarity of the physico-chemical characteristics of Cd and Zn.⁶⁾ However, very little is known about the changes of other metals in the testis induced by Cd exposure. We carried out experiments to clarify how eight kinds of metals, including Zn, are influenced by Cd injection or simultaneous injection of Se and Cd.

Materials and methods. Mature male mice (ICR, 5th wk old) were kept at a constant room temperature ($25 \pm 0.4^\circ\text{C}$) and humidity ($42 \pm 5\%$) and were maintained on a controlled 12 h light-dark illumination cycle. Food (Type MF from Oriental Yeast Co., Ltd., Tokyo) and water were given *ad libitum* to the animals.

Cd (CdCl_2) and/or Se (SeO_3) were administered subcutaneously in deionized water every 24 h for 2 consecutive d. The Cd group was injected with Cd at 0.012 mmol/kg and the Se group, Se at 0.024 mmol/kg. The Cd+Se group was given Cd and Se at the same time but at different sites. The control group was injected only with deionized water.

Blood and organs were obtained 3 d after the second administration. The right testis was fixed in 10% formaldehyde solution at pH 7.5 and stained with hematoxylin-eosin. The other testis, liver and kidney were treated for the measurement of metals by a method previously reported.⁶⁾ A flame (Model 208, Hitachi Corp., Tokyo) or

flameless (Model 180-80, Hitachi) atomic absorption spectrophotometer was used. Phosphorus was measured by the method of Allen.⁸⁾

Results and discussion. In both groups treated with Cd, Cd level of the testis was only 1/20 to 1/10 that of the kidney ($7.3 \pm 0.9 \mu\text{g/g}$ tissue and $8.8 \pm 1.5 \mu\text{g/g}$ in the Cd and Cd+Se groups, respectively) and liver ($18.2 \pm 2.9 \mu\text{g/g}$ tissue and $13.4 \pm 1.3 \mu\text{g/g}$ in the Cd- and Cd+Se-groups, respectively) (Table 1). However, the Cd+Se group showed a value twofold higher than the Cd group alone. This result agreed with that previously reported.⁹⁾

Testicular Zn was significantly decreased by injection of Cd alone (Table 1). With the simultaneous injection of Se, Zn level was returned to the control level. Cu was not changed significantly by Cd injection. Fe was greatly increased by Cd injection. The increase of Fe may be due to the elevation of testicular blood volume, possibly attributable to hemoglobin-Fe.⁷⁾

Of all the elements measured here, Ca showed the highest rate of change following Cd treatment (Table 1). P was decreased rather than increased by this treatment, while Mg was decreased to half the level of the control by Cd injection alone. The changes in levels of two metals, Ca and Mg, suggest that the integrity of the membrane, e.g., the mitochondrial membrane, was lost. With the simultaneous injection of Se, the levels of the above two metals were restored to the control levels.

In a related work, Takkar et al¹⁰⁾ reported previously that a single injection of Cd to rats caused progressively severe calcification of the tubules between 7 and 90 d after treatment and that concurrent administration of Zn and Se prevented Cd-induced calcification of the tubules. Recently, Sajiki et al¹¹⁾ reported that simultaneous injection of Ca antagonist, verapamil, with Cd partly improved Ca channel and then testicular injury. Stacey and Klaassen¹²⁾ showed that changes in Ca flux probably did not play an important role in Cd hepatotoxicity in *in vitro* systems. Under our experimental conditions, it is difficult to determine whether the changes of the two metals proceeded to testicular damage because of the severe and widespread interstitial and tubular damages (photo not shown).

K and Na were changed in a manner similar to Ca and Mg with depression of K and enhance-

Table 1. Element concentration in testis.

Groups	Zn(μ g/g)	Cu(μ g/g)	Fe(μ g/g)	Cd(μ g/10 g)	Ca(μ g/g)	P(mg/g)	Mg(μ g/g)	K(mg/g)	Na(mg/g)
Control(7)	23.5 $\pm$ 1.4	0.83 $\pm$ 0.1	20.0 $\pm$ 1.8	nd	95.7 $\pm$ 19.7	1.4 $\pm$ 0.1	136.5 $\pm$ 9.3	4.3 $\pm$ 0.1	1.0 $\pm$ 0.2
Cd (8)	19.1 $\pm$ 3.4*	0.93 $\pm$ 0.2	58.8 $\pm$ 11.0*	4.7 $\pm$ 0.5	878.1 $\pm$ 352.7*	1.1 $\pm$ 0.3*	79.7 $\pm$ 24.8*	1.5 $\pm$ 0.6*	2.7 $\pm$ 0.3*
Cd+Se (8)	24.5 $\pm$ 1.1	0.92 $\pm$ 0.1	19.7 $\pm$ 3.4	10.7 $\pm$ 1.5	86.2 $\pm$ 20.4	1.4 $\pm$ 0.1	141.5 $\pm$ 5.8	4.5 $\pm$ 0.2*	1.8 $\pm$ 0.2*
Se (7)	21.5 $\pm$ 1.9	0.91 $\pm$ 0.1	18.5 $\pm$ 1.6	nd	80.0 $\pm$ 22.9	1.6 $\pm$ 0.0*	142.5 $\pm$ 1.4	4.5 $\pm$ 0.1*	1.8 $\pm$ 0.3*

Each value represents mean $\pm$ SD (μ g/g, μ g/10 g or mg/g wet tissue). Number of animals is given in parentheses, nd is "not determined". The means of the control and test groups were compared by the two-tailed Student's t-test at the significance level of * p < 0.05.

ment of Na (Table 1). Nechay and Saunderson¹³⁾ reported that Cd inhibited Na-K ATPase *in vitro*. If this effect can be presumed to occur *in vivo*, the low activity of the Na-K pump may be due to conformation changes of this enzyme caused by the binding of Cd. The effective action of Se on the normal regulation of K and Na may result from the improvement of testicular cell damage.

The changes of Ca-Mg and Na-K were not found in the liver and kidney. In both Cd and Cd+Se groups, serum Ca and P were well regulated (data not shown). Serum Cd was $7.3 \pm 4.9 \times 10^{-3}$ μ g/ml and $240.3 \pm 78.5 \times 10^{-3}$ μ g/ml in the Cd and Cd+Se groups, respectively. Gasiewicz and Smith¹⁴⁾ reported that with the simultaneous injection of Cd and Se, each metal showed a high level when compared to that of a single injection of each metal. A certain serum complex bound specifically to Cd and Se seems to decrease the clearance of each element from the blood.

In conclusion, increases or decreases of Ca, Mg, Na, K, Fe and Zn were induced by Cd injection. Following simultaneous Se injection, the changed levels of metals were uniformly returned to the control level. The changes of the concentrations of these metals may be involved in the testicular dysfunction.

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