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SHORT COMMUNICATIONS

Cadmium-induced inhibition of protein secretion from liver: pretreatment effect and specificity among cadmium, copper and zinc

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We previously showed that cadmium (Cd) depresses the activity of serum cholinesterase (CHE; EC 3.1.1.8) at a dose which does not elevate serum levels of leaked enzymes from liver, such as glutamic oxaloacetic transaminase (GOT; EC 2.6.1.1) and glutamic pyruvic transaminase (GPT; EC 2.6.1.2) [1]. This depression was shown not to be caused by direct action of Cd to the secreted enzyme, CHE in serum [1]. Further study revealed that Cd also decreases the concentrations of albumin and total protein in serum [2]. Most of the serum proteins including CHE and albumin are synthesized in the liver and then secreted into the blood stream. Thus, our findings suggest that Cd inhibits the synthesis of secretory proteins and/or the secretory process in the liver at a lower dose that does not cause a leakage of enzymes.

In our previous experiment the activity of serum CHE decreased from the beginning of repeated Cd injections and remained at the low level during the exposure [3]. The CHE activity was recovered soon after the cessation of exposure, while GOT and GPT remained at elevated levels. These observations suggest that the target sites of Cd inhibiting secretory function of the liver are different from those for cell damage that causes enzyme leakage. In the present study we examined the effect of metallothionein (MT) induction on the inhibitory action of Cd because it is reported that Cd-induced liver injury, indicated by an increase in enzyme leakage, was effectively reduced by pre-synthesized MT [4, 5]. The specificity among Cd, zinc (Zn) and copper (Cu) was also examined for inhibition of the secretory function of the liver.

Materials and methods

Animal experiments. Female Wistar strain rats (Jcl, Clea Japan Co., Tokyo) were used at 11–12 weeks of age. Commercial diet (CE-2 diet, Clea Japan Co.) and distilled water were given *ad libitum*.

Solutions for injection were prepared from cadmium chloride, cupric chloride and zinc acetate. To examine the effect of MT induction the animals were pretreated with a single injection of Zn or Cd 24 hr prior to the Cd challenge. For specificity experiments among Cd, Cu and Zn the animals were given a single i.p. injection of the metals. The dose levels for each experiment are given in Tables 1 and 2 and Fig. 1.

Analytical procedures. Serum was separated by centrifugation at 1600 *g* for 10 min. Activities of GOT and GPT and concentration of serum albumin were determined as described previously [2]. Activity of serum CHE was measured using cholinesterase-B-test^{R} (substrate: iodobutyrylthiocholine) (Boeringer Mannheim, Mannheim).

For measurement of metal concentrations each liver sample was acid-digested and analyzed by ICP atomic emission spectrometry (JY48PVH, Seiko Instrument & Electronics, Tokyo).

MT concentration in liver was determined by radioimmunoassay using 160,000 *g* supernatant of the liver homogenate [6].

Statistical analysis. Statistical differences from the control in the pretreatment experiment were evaluated using Welch's *t*-test.

For the specificity experiment alterations of enzyme activities were followed for each animal. Statistical analysis was made by comparing the activities of the same animals between before and after the injection using paired *t*-test [7].

Results and discussion

Serum CHE activity was lowered significantly on day 1 after the Cd challenge in both the saline pre-loaded group (non-pretreated group) and the Zn pre-loaded group (pretreated group) and it had decreased to 41.7 and 41.5% of the control, respectively on day 2 (Fig. 1A). However, serum GPT (Fig. 1B) and GOT (data not shown) activities were not elevated significantly in any groups. MT concentration in the liver was increased to 260 μg/g tissue after 24 hr by pretreatment with Zn (8 mg/kg body wt, i.p.) and Cd accumulated in the liver by a Cd challenge (1.5 mg/kg body wt) was 19.5 μg/g tissue (Table 1). Therefore, it can be estimated that enough MT was induced in the liver to sequester the Cd challenged in the Zn-pretreated group. Nevertheless, preventative effects were not observed for the CHE activity. Rather, Zn also depressed serum CHE activity (Fig. 1A).

The dose, 0.3 mg Cd/kg body wt, used for the Cd pretreatment was the upper limit that did not cause a depression of serum CHE activity, and MT concentration was increased 10-fold higher than the control by the pretreatment (Table 1). However, the pre-synthesized MT was not protective for the Cd-induced inhibition of protein secretion from the liver; the activity of serum CHE and the concentration of serum albumin decreased significantly irrespective of Cd pretreatment (Figs 1C and D). These results suggest that Cd attacks the sites that cannot be protected by MT in the secretory function of the liver and that the target sites are probably different from those causing enzyme leakage.

Table 2 shows the time courses of the activities of serum CHE and GPT for the rats which received a single i.p. injection of saline, Zn, Cu or Cd. Zn is considered to be a less toxic element [8], so higher doses of Zn, compared to Cd, were used in this experiment. On day 2 after Zn injection a dose-dependent depression of CHE activity was observed; the activity was decreased to, on average, 98.9, 83.0, 74.5 and 59.9% of the initial value (on day 0) at doses of 0, 4.0, 8.0 and 16.0 mg Zn/kg body wt, respectively. On the other hand, administration of Zn caused an elevation of GPT activity in serum in a dose-dependent manner. Cu induced a significant decrease in CHE activity only on day 2 (10.4% decrease compared with the initial value), while serum GPT activity was increased 1.8 times higher than the initial value. Administration of Cd at the same dose as Cu caused a significant decrease in serum CHE activity and it was 64.9% of the initial value of day 2. The serum GPT activity was not elevated and, rather, decreased on days 2 and 3.

In summary, MT was not effective in protecting Cd-induced depression of protein secretion from the liver. Both Zn- and Cu-induced depressions of serum CHE activity were accompanied with an elevation of serum activity of a leaked enzyme, GPT, but Cd-induced depres-

Table 1. Effect of Cd pretreatment on liver concentrations of Cd and MT

Treatment		Concentrations (μg/g tissue)	
Pretreatment	Challenge	Cd	MT
Saline	Saline	N.D.*	10 ± 5
Saline	Cd (1.5 mg/kg body wt)	19.5 ± 0.9	327 ± 6
Cd (0.3 mg/kg body wt)	Cd (1.5 mg/kg body wt)	23.3 ± 4.7	362 ± 11
Cd (0.3 mg/kg body wt)		6.0 ± 0.9	104 ± 34

Rats were killed 24 hr after the last injection.
Each value represents the mean ± SD for 5 rats.
* Below detection limit (0.1 μg/g tissue).

Table 2. Changes in activities of serum CHE and GPT with time after a single ip injection of Zn, Cu or Cd

		Time after injection (day)						
Injection		0	0.5	1	2	3	5	7
Saline	CHE	886 ± 276	791 ± 222	839 ± 215	870 ± 180	896 ± 236	816 ± 231	715 ± 146
(N = 5)	GPT	19.0 ± 3.7	21.0 ± 3.8	24.2 ± 3.4*	21.0 ± 5.8	20.4 ± 6.7	21.4 ± 6.7	29.0 ± 6.8*
4 mg Zn/kg body wt	CHE	1035 ± 94	861 ± 133*	782 ± 137**	862 ± 137**	1011 ± 73	1101 ± 184	964 ± 137
(N = 5)	GPT	17.6 ± 1.8	21.8 ± 2.3**	21.0 ± 2.1*	13.4 ± 5.5	24.2 ± 8.4	17.4 ± 3.4	19.8 ± 1.3
8 mg Zn/kg body wt	CHE	995 ± 144	858 ± 164	808 ± 155*	742 ± 125***	912 ± 244	928 ± 101	903 ± 199
(N = 5)	GPT	23.8 ± 4.1	32.8 ± 4.8*	26.8 ± 2.9	15.8 ± 5.9*	15.8 ± 2.4*	23.6 ± 2.7	21.4 ± 7.6
16 mg Zn/kg body wt	CHE	1122 ± 131	845 ± 238	680 ± 109*	572 ± 172**	618 ± 151*	760 ± 260	620 ± 101*
(N = 3)	GPT	24.7 ± 5.0	81.7 ± 15.3*	60.7 ± 0.6*	22.3 ± 2.3	19.0 ± 3.0	25.0 ± 10.8	31.0 ± 6.1
1 mg Cu/kg body wt	CHE	984 ± 360	910 ± 407	819 ± 267	879 ± 339*	996 ± 272	1058 ± 396	1078 ± 488
(N = 5)	GPT	23.6 ± 4.7	41.8 ± 14.4*	31.6 ± 9.2*	28.8 ± 7.6	25.2 ± 5.2	31.4 ± 12.6	25.6 ± 3.8
1 mg Cd/kg body wt	CHE	753 ± 106	520 ± 84*	545 ± 107*	487 ± 73**	528 ± 107*	529 ± 166	512 ± 158
(N = 4)	GPT	28.0 ± 2.0	24.3 ± 3.0	26.3 ± 2.2	22.3 ± 1.7**	23.0 ± 3.7*	27.5 ± 4.8	23.5 ± 3.1

(a) Metals were administered i.p. in a 0.1 ml solution of saline as $Zn(CH_3COO)_2$, $CuCl_2$ or $CdCl_2$.
(b) Blood samples (approximately 0.2 ml) were obtained by cutting the tail of each animal seven times; once before the injection (day 0) and 0.5, 1, 2, 3, 5 and 7 days after the injection under light ether anaesthesia.
(c) Statistically significant differences between before and after the injection are marked as follows; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
(d) The data are expressed as mean ± SD for 3–5 animals.

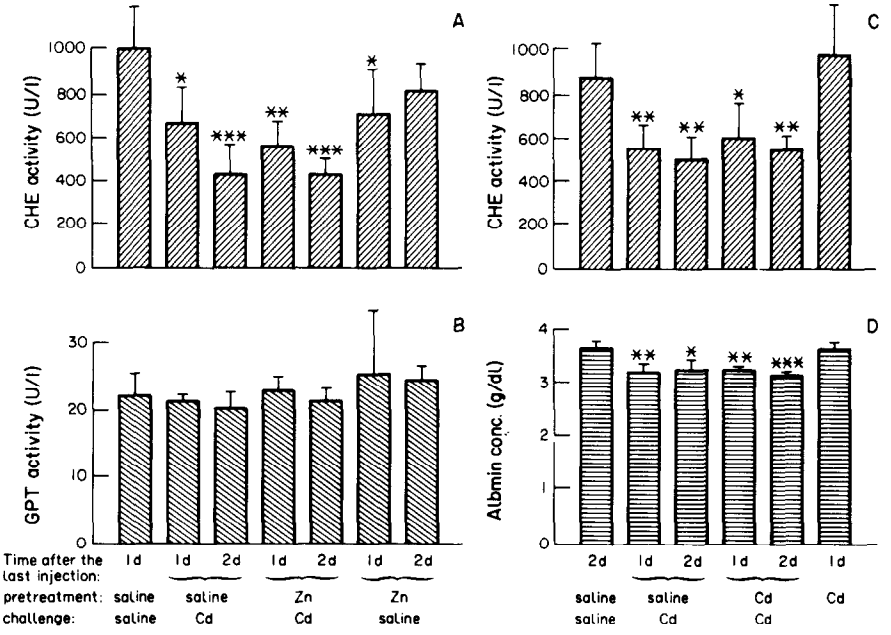

Fig. 1. Effects of pretreatment with Zn or Cd on Cd-induced alterations of serum levels of CHE, GPT and albumin. Rats received the following treatment 24 hr prior to the Cd challenge (1.5 mg/kg body wt, sc): A and B, a single i.p. injection of saline or Zn (8 mg/kg body wt); C and D, a single s.c. injection of saline or Cd (0.3 mg/kg body wt). For comparison two groups of rats pretreated with Zn were injected with saline instead of Cd (A, B) and one group of rats was given Cd pretreatment only (C, D). The control rats were injected with saline twice at 24 hr interval. Data are expressed as means ± SD of 5 samples in each group. *, ** and *** indicate the significant differences from the control at $P < 0.05$, 0.01 and 0.001, respectively.

sion was not accompanied by an enzyme leakage. Thus, the mechanism for the inhibitory action of Cd seems to be different from those of Zn and Cu.

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Effects of ethanol on $^{45}Ca^{2+}$ uptake in synaptosomes and in PC12 cells

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Depolarization-dependent calcium uptake is important in many neuronal functions, such as synaptic transmission [1], and also appears to play an important role in the effects of ethanol. Net $^{45}Ca^{2+}$ uptake into rodent synaptosomes is inhibited in a dose-dependent manner by ethanol *in vitro* [2–5], and after chronic ethanol exposure the potency of *in vitro* ethanol to inhibit synaptosomal Ca^{2+} uptake is reduced [2, 4]. Chronic exposure to ethanol was also reported to decrease net potassium-stimulated $^{45}Ca^{2+}$ uptake in mouse synaptosomes [2], but not in rat synaptosomes [4]. Potassium-stimulated $^{45}Ca^{2+}$ uptake into synaptosomes, however, occurs in a biphasic manner [6].