Effects of Cadmium on Absorption of Copper, Zinc and Iron in Rat Small Intestine *in Vitro*

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The effects of cadmium on intestinal absorption of copper, zinc and iron in rat everted intestine were investigated with and without L-cysteine *in vitro*. Intestinal absorption of copper was inhibited in the cadmium-administered intestine, and was not promoted by L-cysteine. Intestinal absorptions of zinc and iron were clearly inhibited in the cadmium-administered intestine, but were markedly promoted by L-cysteine.

The results of this experiment coincided with those of the previous *in vivo* experiment, in which copper and iron contents in organs were decreased by oral administration of cadmium as compared with those in controls, and the decrease in iron content was suppressed by the simultaneous administration of L-cysteine with cadmium. As regards copper content, the *in vitro* results did not coincide with the *in vivo* findings.

Key words: cadmium; cysteine; copper; zinc; iron; intestinal absorption; everted intestine; ceruloplasmin; metallothionein

Introduction

In the previous studies^{1),2)}, we orally administered cadmium (Cd) alone and Cd plus L-cysteine (Cys) to rats for 20 consecutive weeks to examine the fates of copper (Cu), zinc (Zn) and iron (Fe) in the rats. The results showed that Cd had a marked effect on the fates of the three metals; in particular, the Cu and Fe contents in organs decreased as compared with the control. Such decreases in Cu and Fe contents were suppressed by the simultaneous administration of Cys with Cd. We supposed that the decreases in Cu and Fe contents were attributable to the inhibition of intestinal Cu and Fe absorption by Cd, on the basis of a comparison with the data after subcutaneous administration of Cd.

To confirm this hypothesis, we carried out the present *in vitro* study, in which the effects of Cd on intestinal absorption of Cu, Zn and Fe in rat everted intestine were investigated with and without Cys.

Materials and Methods

1. Preparation of samples for administration and experimental animals

As reported previously¹⁾, samples for administration were prepared, and orally administered to four-week-old Jcl: Sprague Dawley female rats divided into four groups for 4 consecutive weeks.

2. Absorption experiment using everted intestine^{3),4)}

The rats were sacrificed with ether anesthesia at 48 hours after the final administration. The animals had been fasted (but allowed water) for 24 hours before sacrifice. After sacrifice, a section of the small intestine approximately 10 cm in length was quickly withdrawn and cut away from the duodenal intestine. One side of the cut intestine was rapidly ligated with a silk thread, the intestine being everted with a glass bar. The intestinal contents were washed out with cold physiological saline solution. Into the serosa of the everted intestine, 0.6 ml of a reac-

Table 1. Effect of Cadmium on Copper Transport and Accumulation in Rat Small Intestine

Rat small intestine	Mucosal medium	Transported Cu ($\mu\text{g/ml}$ serosal medium)	Accumulated Cu ($\mu\text{g/g}$ wet tissue)
Cd	0.1 mM Cu	0.29 $\pm$ 0.11*	11.7 $\pm$ 3.3*
Control	0.1 mM Cu	0.68 $\pm$ 0.25	28.7 $\pm$ 7.0
Cd	0.1 mM Cu+2.0 mM Cys	0.25 $\pm$ 0.06*	8.6 $\pm$ 1.5*
Control	0.1 mM Cu+2.0 mM Cys	0.86 $\pm$ 0.32	24.8 $\pm$ 4.4

The everted sac was incubated in mucosal medium at 37°C for 60 min.

Cd: CdCl₂·2.5H₂O (80 mg/kg/day) for 4 weeks

Control: Distilled water

Each value is the mean $\pm$ S.D. for 5 intestines.

* $p < 0.05$ from Control

tion solution (serosal medium, 30 mM Tris-hydrochloric acid buffer containing 125 mM sodium chloride and 10 mM fructose, pH 7.4) was injected via an injection syringe with a blunt needle. Immediately after withdrawal of the needle, the intestine was completely ligated with a silk thread. The everted sac was dipped into 10 ml of a reaction solution (mucosal medium) containing 0.1 mM Cu (CuCl₂·2H₂O), 0.2 mM Zn (ZnSO₄·7H₂O) or 0.2 mM Fe (FeSO₄·7H₂O) and another reaction solution containing each of these three metals plus Cys (20 times the molar quantity of metals) in a 25 ml Erlenmeyer flask. Then the sac in the flask was incubated for 60 min at 37°C under oxygen gas. After incubation, the everted sac was taken from the flask, dehydrated, and cut open. The reaction solution in the serosa was transferred into a test tube. The contents of metals in the serosal medium were determined by the direct atomic absorption method after dilution of the medium 1/5 with 0.5 N nitric acid. The contents of metals accumulated in the tissue were determined by the low temperature ashing-atomic absorption method^{8),9)}. Further, to examine the behavior of the metals added to the reaction solution, a blank experiment was performed in which the endogenous metal contents in the intestine were found, then the real content of each metal was obtained by subtracting the endogenous content from the apparent content of each metal.

3. Gel filtration of intestinal mucosal supernatant fraction

After fasting, the rats were sacrificed. A piece of small intestine about 25 cm in length was cut from the duodenal intestine and incised,

the contents being washed out with cold physiological saline solution. Then, the mucosa of the gut was scraped off with a cover glass and homogenized with 5 volumes of ice-cold 0.25 M sucrose solution. The homogenate was ultracentrifuged at 105,000 $\times g$ for 60 min. Then 3 ml of the supernatant fraction was applied to a Sephadex G-75 gel filtration column ($\phi 2.0 \times 60$ cm) and eluted with 0.01 M Tris-hydrochloric acid buffer, pH 7.4, at 30 ml/hr. Fractions of 5 ml were collected.

4. Measurement of ceruloplasmin (CP) activity and Cu content in rat sera

Serum CP activity was measured by the method using Bandrowski base as a standard substance^{7),8)}, and serum Cu content by the direct atomic absorption method after dilution of the serum 1/10 with 0.5 N nitric acid.

Results

1. Effects of Cd on intestinal absorption of Cu

When Cu concentration in mucosal medium was set at 0.1 mM and the medium was incubated for 60 min, the Cu concentration in serosal medium of the Cd-administered intestine was about less than half that of the control. When the mucosal medium was incubated with Cys at 20 times the molar concentration of Cu, the Cu concentration in the serosal medium was nearly the same as that obtained without Cys, in both the Cd-administered intestine and the control. The accumulated Cu content in the small intestinal tissue of the Cd-administered intestine decreased to less than 1/2 that in the control, irrespective of the addition of Cys (Table 1).

Table 2. Effect of Cadmium on Zinc Transport and Accumulation in Rat Small Intestine

Rat small intestine	Mucosal medium	Transported Zn ($\mu\text{g/ml}$ serosal medium)	Accumulated Zn ($\mu\text{g/g}$ wet tissue)
Cd	0.2 mM Zn	1.8 $\pm$ 0.4*	79.7 $\pm$ 21.2*
Control	0.2 mM Zn	7.0 $\pm$ 1.8	241.6 $\pm$ 46.9
Cd	0.2 mM Zn+4.0 mM Cys	7.0 $\pm$ 2.1*	76.7 $\pm$ 13.7*
Control	0.2 mM Zn+4.0 mM Cys	15.1 $\pm$ 3.2	202.7 $\pm$ 31.5

The everted sac was incubated in mucosal medium at 37°C for 60 min.

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg/day) for 4 weeks

Control: Distilled water

Each value is the mean $\pm$ S.D. for 5 intestines.

* $p < 0.05$ from Control

Table 3. Effect of Cadmium on Iron Transport and Accumulation in Rat Small Intestine

Rat small intestine	Mucosal medium	Transported Fe ($\mu\text{g/ml}$ serosal medium)	Accumulated Fe ($\mu\text{g/g}$ wet tissue)
Cd	0.2 mM Fe	0.15 $\pm$ 0.09*	36.3 $\pm$ 4.6*
Control	0.2 mM Fe	0.44 $\pm$ 0.15	102.3 $\pm$ 9.4
Cd	0.2 mM Fe+4.0 mM Cys	7.2 $\pm$ 1.3*	39.5 $\pm$ 8.3*
Control	0.2 mM Fe+4.0 mM Cys	17.0 $\pm$ 1.7	121.0 $\pm$ 15.7

The everted sac was incubated in mucosal medium at 37°C for 60 min.

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg/day) for 4 weeks

Control: Distilled water

Each value is the mean $\pm$ S.D. for 5 intestines.

* $p < 0.05$ from Control

2. Effects of Cd on intestinal absorption of Zn

When the mucosal medium was incubated at a Zn concentration of 0.2 mM for 60 min, the Zn concentration in serosal medium of the Cd-administered intestine decreased to about 1/4 that of the control. After incubation with the addition of Cys, the amount of transported Zn increased about 4 times in the Cd-administered intestine, and about 2 times in the control as compared with the non-addition of Cys. However, the amount of transported Zn in the Cd-administered intestine was approximately 1/2 that in the control. The content of accumulated Zn in the small intestinal tissue of the Cd-administered intestine decreased to about 1/3 that in the control, irrespective of the addition of Cys (Table 2).

3. Effects of Cd on intestinal absorption of Fe

When the mucosal medium was incubated at an Fe concentration of 0.2 mM for 60 min with and without Cys, the amount of transported Fe varied as seen in the case of Zn. The amounts of accumulated Fe in the small intestinal tissue with and without Cys also varied as observed

Fig. 1. Sephadex G-75 gel filtration of intestinal mucosa supernatant after repeated oral administration for 4 weeks

(A): $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg)

(B): control(H_2O)

—○—: Cd; —●—: Zn

—△—: Cu; —▲—: Fe

Table 4. Ceruloplasmin Activity and Cu Content in Rat Sera After Repeated Oral Administration of Cd and L-Cysteine for 4 Weeks

	Cd	Cd+Cys	Control	Cys
Ceruloplasmin (mU/ml)	24.0 $\pm$ 9.0*	71.0 $\pm$ 22.4**	80.3 $\pm$ 5.1	88.6 $\pm$ 9.3
Cu (μ g/ml)	0.39 $\pm$ 0.15*	1.10 $\pm$ 0.31**	1.41 $\pm$ 0.06	1.35 $\pm$ 0.11

Cd: CdCl₂·2.5H₂O (80 mg/kg); Cd+Cys: CdCl₂·2.5H₂O (80 mg/kg) + cysteine·HCl (1,500 mg/kg)

Control: distilled water; Cys: cysteine·HCl (1,500 mg/kg)

Each value is the mean $\pm$ S.D. for 5 rats.

* $p < 0.05$ from control

** $p < 0.05$ from Cd

in the case of Zn (Table 3).

4. Gel filtration of intestinal mucosal supernatant fraction

The intestinal mucosal supernatant fraction from the rats administered Cd alone for 4 weeks was subjected to Sephadex G-75 gel filtration to examine the distribution of metals (Fig. 1).

Most of the Cd in the supernatant fraction was present in the metallothionein (MT) fraction. Cu was not as extensively distributed in the MT fraction as in the case of the control, while Zn was increased in the MT fraction. Fe was decreased in the high molecular fraction (apo-ferritin fraction) as compared with the control.

5. CP activity and Cu content in sera after repeated oral administration

Serum CP activity and Cu content were examined in four groups orally given Cd only, Cd plus Cys, distilled water and Cys only for 4 consecutive weeks (Table 4).

The Cd group showed significantly low CP activity and Cu content as compared with the control group, while the Cd plus Cys group showed slightly (but not significantly) lower CP activity and Cu content as compared with the Cys group.

Discussion

In the previous reports^{1),2)}, it was proved that Cd greatly affected the fates of Cu, Zn and Fe. In particular, the Cu and Fe contents in organs decreased as compared with the control value. It was also found that the simultaneous administration of Cys with Cd suppressed such decreases in Cu and Fe contents. We supposed that these phenomena might occur at the intestinal absorption level, and so we performed the present study to examine this hypothesis in

an *in vitro* experiment using rat everted intestine. Although there are many reports on the transport capabilities of calcium⁴⁾ and Cd⁹⁾⁻¹¹⁾ in everted intestine, little is known about the transport capabilities of Cu, Zn and Fe.

As regards the effects of Cd on intestinal Cu absorption, Cd clearly inhibited Cu absorption, but Cys did not promote Cu absorption (Table 1). The former result is consistent with the previous finding¹⁾ that oral administration of Cd decreased the Cu content in organs as compared with the control. The latter is not consistent with the previous finding that the simultaneous administration of Cys with Cd suppressed such a decrease in Cu content. Thus, serum CP activity and Cu content were examined in rats orally administered Cd only, Cd plus Cys, distilled water and Cys only for 4 consecutive weeks. The results indicated inhibition of Cu absorption by the administration of Cd and promotion of Cu absorption by the simultaneous administration of Cys with Cd (Table 4). Accordingly, it seems that the *in vitro* experimental results do not reflect the *in vivo* findings. Very little is known about the intestinal absorption of Cu. Kojima *et al.*¹⁰⁾ reported that the higher the stability constant of an amino acid with Cd, the better the amino acid promotes intestinal transport of Cd. The stability constant between Cys and Cu is 19.2, which is far higher than that of Fe (6.2) and ranks with that of Zn (18.7). Therefore, it seemed that intestinal transport of Cu was promoted by Cys in the same way as in the cases of Zn and Fe (Tables 2 and 3). However, the *in vitro* experimental results did not accord with this supposition. There is evidence of an active transport system for Cu which presumably exists at the membrane on the serosal aspect of the cell.¹²⁾ Possibly Cys did

not promote the transport of Cu because an active transport process was not produced in consequence of the use of the everted intestine system *in vitro*.

As regards the effects of Cd on intestinal Zn and Fe absorptions, Cd evidently inhibited both Zn and Fe absorptions. Cys markedly promoted both Zn and Fe absorptions (Tables 2 and 3). The results concerning Zn in this study are consistent with the supposition based on the previous findings¹⁾ that the Zn content in organs is increased rapidly by oral administration of Cd, then gradually decreased. Therefore, Cd may inhibit Zn absorption. Further, the Zn content in organs was not decreased whereas the Zn content in urine was increased by Cys, so that Cys may promote Zn absorption. With regard to Fe, the results of this study are consistent with the previous findings²⁾ that the Fe content in organs was markedly decreased by oral administration of Cd as compared with the control value, and that such a decrease in Fe content was suppressed by the simultaneous administration of Cys with Cd.

The data indicated that Cd inhibited Cu, Zn and Fe transports, because the amounts of metals (Cu, Zn and Fe) absorbed or adsorbed on the Cd-administered rat intestine were evidently lower than those in the control, and accordingly, the amounts of transported metals were probably decreased. In the gel filtration profile of the intestinal mucosal supernatant fraction shown in Fig. 1, a large portion of Cd is observed in the MT fraction in the Cd-administered rats. Based on the mean weight of the everted sacs (each about 5–6 cm in length), 0.605 g for Cd-administered intestine and 0.380 g for controls, it was apparent that Cd-administered intestine did thicken or enlarge. As a related matter, it has been reported that the intestinal function was impaired by Cd^{13),14)}. This may explain why the absorption or adsorption of metals (Cu, Zn and Fe) on Cd-administered intestine was apparently decreased as compared with that of the control.

Kojima *et al.*^{9),10)} reported that coexistence of a chelating agent such as EDTA, L-cysteine, L-histidine or other amino acids promoted intestinal Cd absorption. The reason was supposed to be that Cd might pass through the epithelial cells of

the intestinal mucosa after forming a stable compound with a chelator. In this study as well, coexistence of Cys increased intestinal absorptions of both Zn and Fe, presumably through a mechanism similar to that proposed by Kojima *et al.*

Many studies have been carried out on the interaction between Cd and other metals (Cu, Zn and Fe), especially on the effects of Cd on intestinal absorption of essential metals, and the inhibition of intestinal Zn and Fe absorptions by Cd has been suggested by *in vivo*^{15),16)} and *in situ*¹⁷⁾ experiments. Similar results were obtained from our *in vitro* experiment using rat everted intestine. Thus, this experimental system should be very useful for studies on intestinal absorption and on the fates of Cu, Zn and Fe after oral administration of Cd.

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