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Interactions of Cadmium Compounds with Endogenous Iron in the Intestinal Tract

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According to the hypothesis proposed by Harriss and Hohenemser (1978), Cd is classified as the second most dangerous metal in our environment. Furthermore, we can now detect the metal in natural food, for example, rice, oysters, mushrooms, cattle liver and kidney (Fassett 1980). This implies that Cd can enter the body via the gastrointestinal (GI) tract, and that its risk should be surveyed at all times.

We (Sugawara et al. 1984, 1988) previously reported that when Cd was given orally to mice or rats, they showed a decrease of hemoglobin, or of hepatic and renal Fe. The decrease may be due to the decrease of Fe uptake into the intestinal mucosa brush border membrane (Hamilton and Valberg 1974). In a related work, Huebers et al. (1987) suggested that internalized-Cd blocks the transferrin cycle within intestinal cells. However, Sugawara et al. (1988) reported later that transferrin formation was not prevented in the duodenal mucosa cells of rats treated orally with $CdCl_2$.

The role of transferrin in Fe absorption was proposed previously by Huebers et al. (1983). Recently, Ehtechami et al. (1989) re-evaluated the role of ferritin in the process of intestinal Fe absorption. Even now, Fe absorption from the GI tract is still under discussion. In order to understand the competition of Cd with Fe further, we gave some Cd compounds known to be taken up in different manners into the intestinal mucosa to mice.

MATERIALS AND METHODS

ICR strain male mice aged 6 weeks old were used. The food and tap water contained a 140 μg/g diet of Fe and 0.05 μg/ml of Fe, respectively. Cadmium chloride solu-

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tion containing 10 or 50 μg of Cd was intubated orally with a capillary to the upper part of the duodenum under light ether anesthesia (Table 1). Mice were fasted for 12 hr before the intubation. After further starvation with only water for 6 hr, they were sacrificed.

The small intestine (15-20 cm from the pylorus) was removed from the body, cut longitudinally and then washed well in 0.9% solution. Half of the intestine was digested with a mixture of nitric and perchloric acid to measure metal concentration. The other half was homogenized using a Polytron with 10 volumes of 0.25 M sucrose solution to determine metallothionein (MT) or ferritin-bound Fe concentrations (Table 1).

A second experiment was carried out (Table 2 and Fig. 1). Male mice were divided into 4 groups. The Cd-sc group received a single subcutaneous injection of Cd (1.0 mg/kg) as $CdCl_2$. Mice were sacrificed 24 hr after the injection. Animals were deprived of food for the last 6 hr. The Cd-or group was orally given Cd (50 μg) as $CdCl_2$ with a capillary. The Cd-cy group was orally given a mixture of Cd (50 μg) as $CdCl_2$ and cysteine (746 μg). The ratio of Cd/cysteine was 1/14 (mol/mol). Mice in the Cd-or and Cd-cy groups were killed 6 hr after intubation. No food was given during the 6 hr. The control group was orally given only 0.1 ml of deionized water. The small intestines (15-20 cm) were removed from these mice and the tissues were used for measurement of metals and MT. Metal was measured using a Hitachi 208 atomic absorption spectrophotometer (AAS) with an air/acetylene flame or a Hitachi 180-80 with a graphite furnace.

A third series was studied (Table 3 and Fig. 2). Male mice were divided into 4 groups. The Cd and MT groups were orally given Cd (25 μg) as $CdCl_2$ and Cd-MT (MT-II isolated from rat liver), respectively. The intubated MT solution contained 25 μg of Cd. The CdCy group was orally given a mixture of Cd (25 μg) as $CdCl_2$ and cysteine (43.8 μg). The ratio of Cd/cysteine was about 1/6 (mol/mol). The control group was given only water.

Intubation was done twice every 24 hr to the upper part of the duodenum. Mice were sacrificed 24 hr after the second dose. For the last 6 hr, they were deprived of food. The small intestine was removed and homogenized. After centrifugation (31,000 g for 40 min) of the homogenate, the supernatant was applied to a Sephadex G-75 column (Fig. 2). Protein was determined by the method of Lowry et al. (1951). Statistical analysis was performed by Student's t-test or analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

RESULTS AND DISCUSSION

Intestinal Fe concentrations decreased 6 hr after the oral intubation of Cd, especially in the Cd-50 group (Table 1). The decrease of total Fe was accompanied by a decrease of ferritin-Fe (Table 1). No F-ratio obtained by the ANOVA test had a statistically significant Fe or ferritin-bound Fe concentration. When the data of the two Cd groups were combined for comparison to that of the control group, the difference of means between the combined- and control groups was significant for intestinal Fe ($p<0.05$) and ferritin-bound Fe ($p<0.05$) concentrations. However, there was no significant difference in the hepatic Fe concentration. Sugawara et al.(1984) previously reported that intestinal Fe was decreased by long exposure of mice to Cd. The present results (Table 1) suggest that the decrease of Fe is due to the prevention of Fe uptake at the mucosal sites.

For the intestinal Cd and MT concentrations, the Cd-50 group showed higher values than the Cd-10 group did (Table 1). In the two groups, there was a negative correlation coefficient ($r=-0.523$, $Y=8.132-0.194X$, $n=10$) between Fe(X) and Cd(Y) concentrations. The results strongly indicated that the two metals were taken up competitively at the intestinal mucosa. As shown above, however, the decrease of intestinal Fe did not yet influence the Fe concentration in the liver (Table 1). Hepatic Cd concentration was about 10 times higher in the Cd-50 group than in the Cd-10 group. There was no significant relation between hepatic Cd and Fe.

As for the intestinal Fe concentration, the Cd-po group showed a significantly low concentration of Fe compared to the other groups (Table 2). In the Cd-sc group, the decrease of Fe was not significant compared to that in the control group (Table 2). Although there was no difference in Cd and MT concentrations between the Cd-po and Cd-cy groups, a significant difference was found in the Fe concentration. Accordingly, we can suggest two possibilities. One is that ionic Cd is an integral form for the inhibition of Fe uptake and that the internalized Cd does not affect the uptake of lumenal Fe into the intestine. More probably, lumenal Cd ions compete with Fe at the brush border membrane (BBM) to decrease the uptake of Fe into the intestine. The decrease may bring about the reduction of hepatic or renal Fe and, eventually, cause anemia.

Table 1. Concentrations of Fe, ferritin-bound Fe, Cd and metallothionein in the intestine, and of Fe and Cd in the liver

		Intestine				Liver	
		Fe	Ferritin-Fe	Cd	MT	Fe	Cd
		(μg/g tissue)	(μg/g protein)	(μg/g tissue)	(μg/g protein)	(μg/g tissue)	(μg/g tissue)
Control	(4)	32.33±3.04	242.0±85.0	-a	-a	214.2±23.7	-
Cd-10	(5)	29.26±3.30	157.0±35.0	1.80±0.74	50.2±23.2	202.2±48.8	0.084±0.07
Cd-50	(5)	28.20±1.82	167.0±29.0	3.37±0.62	77.2±11.7	257.4±63.5	0.830±0.27

The ferritin fraction was isolated by the method of Vidnes and Helgeland (1973). MT was determined by a modification of the method of Cd/hemoglobin affinity, followed by filtration through a Millipore filter (pore size, 0.05 μm) (Sugawara and Sugawara 1982). The F-ratio obtained from ANOVA was not significant for intestinal Fe, ferritin-Fe and hepatic Fe concentrations. Ferritin-Fe and MT are expressed as Fe and Cd μg/g protein, respectively.
a: not determined.

Table 3. Concentrations of Fe, Cd and MT in the intestinal supernatant, and of Fe and Cd in the liver

		Intestine			Liver	
		Fe	Cd	MT	Fe	Cd
		(μg/g protein)	(μg/g protein)	(Cd μg/g protein)	(μg/g tissue)	(μg/g tissue)
Control	(5)	176.63±11.8	-a	34.91±13.8c	185.3±31.3	0.020±0.00
Cd	(7)	123.38±21.3b,c	9.164±2.0	120.27±24.0b	231.3±50.2	0.698±0.44d
MT	(7)	212.72±38.3	3.152±1.0d	93.70±19.6	224.1±23.2	0.079±0.03
CdCy	(8)	178.63±29.0	11.889±3.9	118.59±23.5	229.9±47.7	0.347±0.24

a: not determined. b: 6 mice. c: significant at $p<0.05$ compared to the other three groups. d: significant at $p<0.05$ compared to the other groups except for the control.

Table 2. Concentrations of Cd, MT and Fe in the intestine

		Intestine		
		Cd (μg/g tissue)	MT (Cd μg/g tissue)	Fe (μg/g tissue)
Control	(5)	-a	-a	29.72±3.3
Cd-sc	(6)	1.40±0.1b	-a	25.90±4.0c
Cd-or	(7)	3.63±0.8	7.21±2.3	21.72±1.5d
Cd-cy	(7)	3.15±0.5	9.85±1.5	27.02±3.3

a: not determined. b: significant at $p<0.05$ by Dunnett's test compared to the Cd-or or Cd-cy group. c: 5 mice. d: significant at $p<0.05$ compared to the control or Cd-cy group.

When Cd as $CdCl_2$ was given subcutaneously, Cd could be detected at a low concentration in the intestine (Table 2), but in the liver the Cd concentration (1.17±0.61 (M±SD) μg/g liver) was significantly higher than that of the other two groups (0.15±0.05 and 0.53±0.38 μg/g liver in the Cd-or and Cd-cy groups, respectively). Unfortunately, the MT concentration in the intestine could not be determined. However, about 35% of the Cd in the intestinal supernatant was located in the MT region on the Sephadex G-75 column (figure not shown).

Intestinal ALPase activity was decreased with increasing Cd concentrations (Fig. 1). The high activity of ALPase in the Cd-sc group may have been due to its low concentration of Cd. These results indicated that the status of Cd in the intestine of the Cd-sc group was very similar to that in the Cd-or group.

The intestinal Fe concentration was significantly lower in the Cd group than in the other groups, including the control (Table 3). In particular, although there was no difference in Cd and MT concentrations between the Cd and CdCy groups, the Fe concentration was significantly lower in the Cd group than in the CdCy group. Furthermore, the MT group did not show any downward trend of Fe concentration (Table 3). Actually, in the MT group, the Cd concentration was approximately 1/3 of that in the Cd and CdCy groups, although the MT concentration was not different from either one. These results imply that the three lumenal Cd compounds were taken up into the intestinal mucosa in different manners. Cysteine-bound Cd and, probably, MT-bound Cd were taken up in inact form.

The control group showed the lowest mean hepatic Fe concentration of the four groups (Table 3). However, there were no statisticaly significant differences

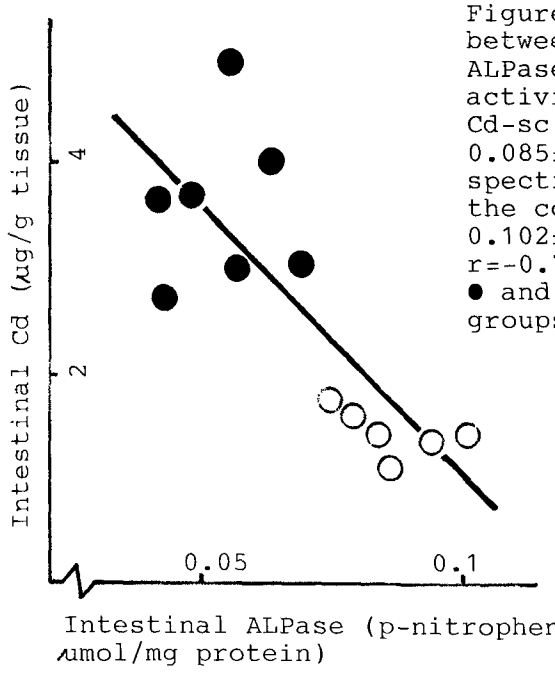

Figure 1 Relationship between intestinal Cd and ALPase. The means of the activity in Cd-or and Cd-sc were 0.054±0.01 and 0.085±0.01 (M±SD), respectively. The mean of the control group was 0.102±0.01. Coefficient: $r=-0.716$ ($n=13$, $p<0.01$). ● and O: Cd-or and Cd-sc groups, respectively. ALPase (E.C.3.1.3.1) was determined by the method of Bessey et al.(1946).

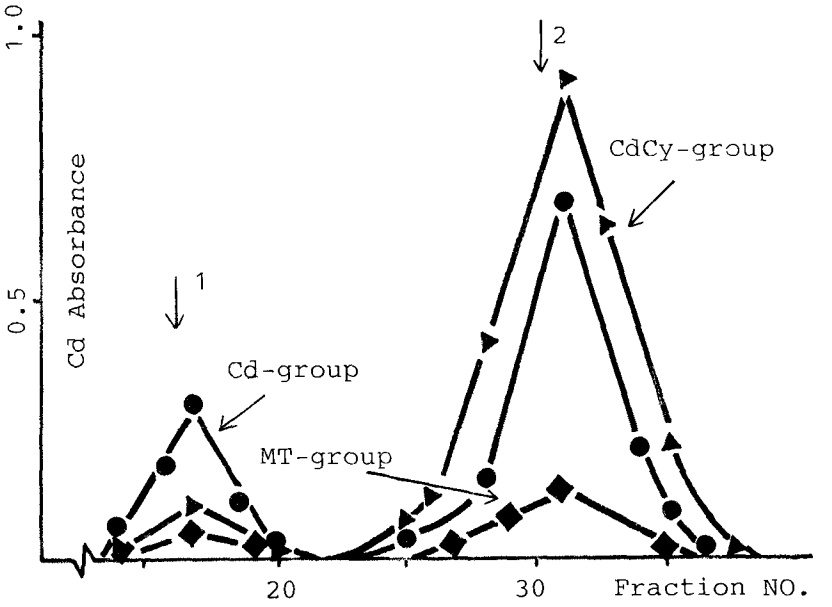

Figure 2 Distribution of Cd in the intestinal supernatant. Intestinal supernatant was applied to a Sephadex G-75 column (1.0x45 cm) equilibrated with 0.02M Tris-HCl buffer (pH 8.5). Markers 1 and 2 are the elution points of blue dextran and cytochrome C, respectively.

among these groups. Hepatic Cd accumulated to the greatest concentration in the Cd group, followed by the CdCy- and MT-groups (Table 3). These results suggest that these three Cd compounds are taken up into the intestine not only in different ways, but also that they circulate in the blood in different ways.

Although competition between lumenal Cd and Fe has been reported (Pond and Walker 1972; Freeland and Cousins 1973), its mechanism, especially in the absorption cells, is still uncertain. Recently, Huebers et al. (1987) noted that Cd in some way blocks the transferrin cycle within the mucosal cells. Their report suggests that the internalized-Cd in the mucosal cells depresses the transfer of Fe to the body. However, our results obtained from administration of a Cd-cysteine complex (Tables 2 and 3) and Cd-MT (Table 3) did not support their hypothesis.

It is known that Cd is bound specifically to MT protein (Sugawara and Sugawara 1987), and that Fe is bound mainly to ferritin and transferrin (Worwood and Jacobs 1971). In this study, Cd was distributed in the MT fraction, regardless of the Cd compound (Fig. 2). Even when Cd was given subcutaneously, it was found in the intestine at a low level (Table 2). This suggests that even when Cd is given parenterally, anemia or depression of tissue Fe due to a deficit of Fe absorption should be observed. However, previous reports (Sugawara et al. 1984; Prigge et al. 1977) do not support this hypothesis. Therefore, the further transfer through the basolateral membrane may be different between Cd and Fe, although the two metals may share common sites for their uptake into the mucosa BBMs (Valberg 1976).

In Fe deficiency, absorption of Cd is stimulated (Hamilton and Valberg 1974). Accordingly, females who have latent Fe deficiency should avoid exposure to Cd, especially oral ionic Cd. Thus, Cd exposure for a long period presents a possible risk for anemia.

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