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Effect of Cadmium on Fe^{+3}-transferrin Formation in the Rat Intestinal Mucosa

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The effect of Cd on Fe metabolism has been an important subject for Cd toxicity, since anemia is usually observed in Itai-Itai patients who are exposed for long periods to Cd from the surrounding environment (Nogawa et al 1979). It was previously accepted that Cd-induced anemia was not dependent on the route of administration (Berlin and Frieberg 1960; Axelsson and Piscator 1966). Thereafter, however, Prigge et al (1977) showed that oral Cd administration was essential for the development of anemia. Sugawara et al (1984) found that the stored Fe was markedly depressed by the ingestion of Cd but not by its injection. These studies suggest that one of the possible sensitive sites of competition between Cd and Fe is in the gastrointestinal tract.

Hamilton and Valberg (1974) reported that Cd competes with Fe at one or more steps in the transport system and that these metals undergo the same step(s) during their absorption. Their hypothesis implies that these two metals possess a common carrier. In the mucosa, Fe is bound to some proteins such as ferritin, transferrin and to some unidentified proteins (Worwood and Jacobs 1971). Huebers et al (1979) reported that Cd is associated with mucosal ferritin in vivo. However, in the mucosa of rats fed with Cd, Cd is predominantly found in the metallothionein (MT) protein (Sugawara and Sugawara 1987)

Two Fe-binding proteins, ferritin and transferrin are well documented in the case of Fe deficiency but not in Cd exposed animals. Recently, we (Sugawara and Sugawara 1987) reported that the status of mucosal Fe-binding proteins in rats fed with Cd was similar to that in the Fe deficient rats. The present work was performed in an attempt to clarify the effect of in vivo and in vitro

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Cd on mucosal transferrin formation,which is one of two main Fe-binding proteins.

MATERIALS AND METHODS

Specific pathogene-free male Wistar rats (6 weeks old) were housed under a 12-hr light-dark cycle (7.00 pm-7.00 am). After one week of acclimatization, the animals were randomly assigned to one (8 rats) of the two groups. The Cd group received tap water containing 100 ppm Cd ($CdCl_2$) for 30 days. A bottle of water (200 ml volumes) having one drop of CH_3COOH was prepared every day. The control group was given acidic tap water without Cd. The water and food (MF-type food obtained from Oriental Yeast Company, Tokyo, Japan) was given ad libitum. The food contained Fe of 140 μg/g, well over the recommended level (35 μg/g) by the NRC. After 30 days, they were killed 12 hr after starvation. Small intestines (about 50 cm from the pylorus), liver, kidney and spleen were excised from the rats of each group.

The mucosa was scraped off with a glass slide on a chilled glass plate. After weighing, the mucosa was divided into two parts. One part was digested with the mixture of nitric and perchloric acids to measure Fe and Cd concentrations. The liver, kidney and spleen were also digested with the acid-mixture to estimate Fe status in the body. Another part of the mucosa was homogenized with 10 volumes of 0.25 M sucrose solution (pH 7.4) by a teflon-glass homogenizer. The homogenate was centrifuged twice at 105,000 g for 60 min at 4°C. The upper fraction was used for the assay of transferrin formation (Tophan et al 1981). The method is based on the conversion of apo-transferrin to transferrin (Δabsorbance at 460 nm). The remaining cytosol was applied to a Sepharose 6B column (1.5x60 cm eluted with 0.054 M triethanolamine-NaOH buffer pH 7.2) for separation of Fe-binding proteins.

The Fe and Cd concentrations were measured with a flame (Hitachi 208 type, Tokyo, Japan) or flameless (Hitachi 180-80 type) atomic absorption spectrophotometer. Protein concentration was estimated by the method of Lowry et al (1951). Hemoglobin was assayed by the cyanmethemoglobin method. Cd-metallothionein(II)(-MT(II)) was isolated from rat liver and then displaced with excess Cd in vitro. Its ratio of A_{254}/A_{280} was 12.5. Results were analyzed by Student's t-test with $p<0.05$ as a limit of significance.

RESULTS AND DISCUSSION

At the end of experiment, a mean of body weights reduc-

ed slightly in the Cd group (173±3 g → 288±10 g) compared to the control group (169±4 g → 322±18 g). Exposure of Cd at 100 ppm markedly decreased the stored Fe concentration in the organs (Table 1) and in addition significantly decreased the hemoglobin concentration (Table 1). The Cd group rats became anemic. The decrease of stored Fe and hemoglobin may be due to the decrease of Fe absorption from the gastrointestine (Table 2), which had been supported by several reports previously published (Pond and Walker,Jr 1972; Freeland and Cousins 1973). Approximately 80% of mucosal Cd existed in the cytosol fraction (Table 2), in the form of MT (data not shown). On the contrary, Fe was at only the 30% level in each group. The results suggest that Cd prevents the uptake of Fe into the whole mucosa.

Table 1 Hemoglobin and Fe concentration in organs

Group	Hemoglobin g/dl	Fe concentration (μg/g) Liver	Kidney	Spleen
Control (8)	14.4±0.4	76.9±8.8	50.3±2.2	262.7±47.8
Cd (8)	*10.9±1.2	*25.3±2.1	*29.8±7.5	*129.1±13.8

Fe: μg/g wet weight. Data: (mean±SD). *p<0.05

Table 2 Concentrations of Fe and Cd in mucosa

Group	Whole Mucosa Fe	Cd	Mocosal Cytosol Fe	Cd	ratio of cytosol/whole Fe	Cd
Control	23.7±6.0	-	8.3±6.1	-	0.30	-
Cd	*15.2±1.5	9.6±1.7	*4.8±1.9	7.7±1.4	0.31	0.79

Fe and Cd: μg/g wet mucosa. Data: mean±SD of 8 rats in each group. *p<0.05.

In the cytosol fraction,three large peaks of Fe-binding proteins could be detected (Fig. 1). Cd exposure decreased Fe content in these peaks (Fig. 1). In Fe deficiency, virtually no mucosal ferritin can be demonstrated, but the amounts of mucosal transferrin and the activity of the Fe transport system within the mucosa is greatly increased (Savin and Cook 1977,1980). Recently, however, we (Sugawara and Sugawara 1987) reported that ^{59}Fe absorption from the intestinal tract was by no means enhanced in anemic rats caused by Cd. These facts suggest that Fe deficiency caused by Cd is different from simple Fe deficiency.

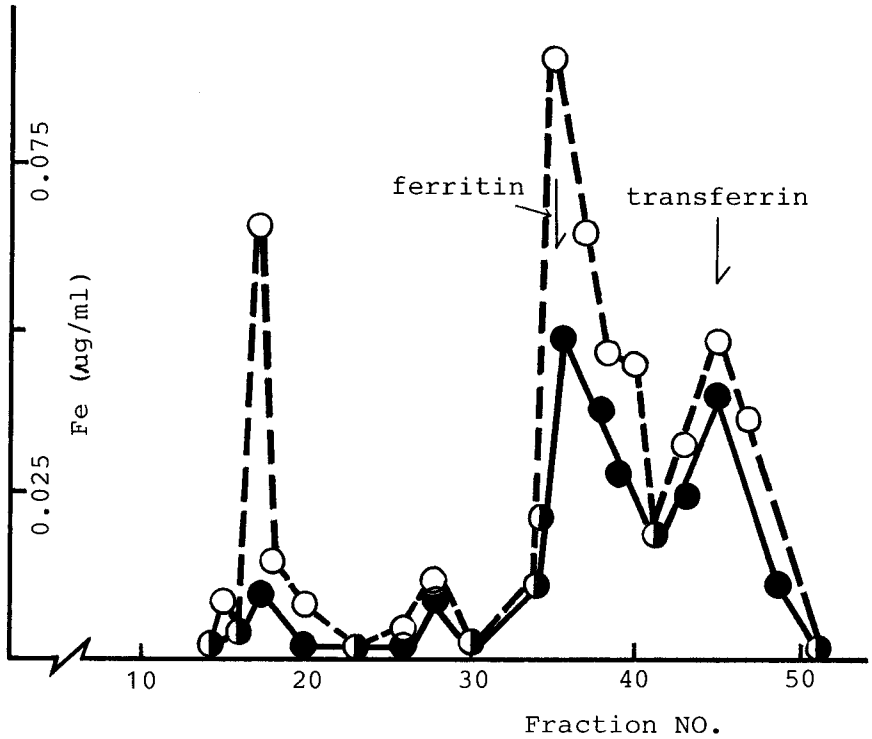

Figure 1. Distribution of Fe-binding proteins in mucosa. The cytosol of the control(O—O) and Cd (●—●) groups were applied to a Sepharose 6B column with the same protein amounts. Two ml volumes were collected.

Table 3 Transferrin formation in mucosal cytosol

	Transferrin
Control-group (8)	0.558±0.063
Cd- " (8)	*0.643±0.049

Transferrin concentration: Δ absorbance (O.D.) at 460 nm/mg protein. Data: mean±SD. *$p<0.05$.

In Table 3, the activity of transferrin was significantly higher in the Cd group than in the control group. Furthermore, in vitro $CdCl_2$ inhibited only slightly the conversion of apo-transferrin to transferrin even at a high Cd concentration (Fig. 2). Cd-MT (II) had no effect on this step (Fig. 2). These results suggest that the conversion step is not involved in Fe deficiency causd by Cd.

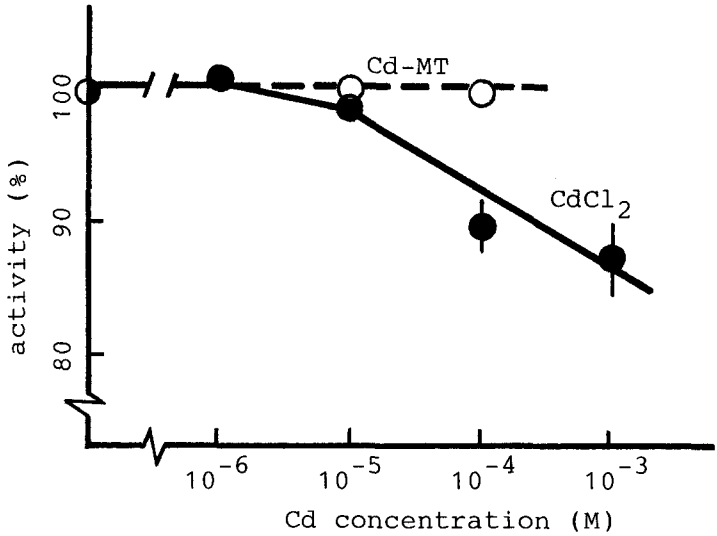

Figure 2. Effect of in vitro $CdCl_2$ and Cd-MT on transferrin formation. The reaction mixture was composed of 0.2 ml 0.6 M acetate buffer pH 6.0, 0.2 ml $4x10^{-4}$ M ferrous sulfate, 0.1 ml 2% apo-transferrin, 50 µl mucosal cytosol (0.18 mg protein) from the control rat and $CdCl_2$ or Cd-MT solution. This mixture was incubated for 30 min at 30°C. After incubation, absorbance at 460 nm was measured. Activities (mean±SE of 2-8 assays) at each Cd concentration were calculated as % of the control.

Recently, Huebers et al (1987) reported that Cd inhibited the absorption of transferrin-Fe but not of hemoglobin-Fe and then Cd increased the content of transferrin-Fe in the mucosa. The results indicate that Cd in some way blocks the transferrin cycle within the cell. In their experiment, mucosal Cd existed mainly in the ferritin but not MT in normal rats exposed once to Cd. This observation was completely different from our previous result (Sugawara and Sugawara 1987) observed in almost the same procedures as described in this paper.

As mentioned by Savin and Cook (1980), two compartments being measured by mucosal ferritin and transferrin correlates closely with Fe absorption and body Fe status. The role of mucosal ferritin or other unidentified Fe-binding proteins should be reevaluated in the Fe deficiency caused by Cd exposure. It was concluded that oral Cd causes anemia even in experimental animals provided with sufficient Fe.

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