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Role of Mucosal Metallothionein Preinduced by Oral Cd or Zn on the Intestinal Absorption of a Subsequent Cd Dose

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Intestinal absorption of Cd is one of the main channels of Cd entry into the body leading to systemic toxicity. When Cd is absorbed, it first comes into contact with the gastrointestinal mucosa, particularly the duodenum mucosa. Therefore, the mucosal behavior of Cd is one of the most important factors, not only for determining the absorption rate, but also as concerns the interactions between Cd and other metals.

The toxicological importance of metallothionein (MT), specifically with regard to its function as a defense mechanism, has been well documented (Webb and Can 1982). However, the role of MT on Cd absorption is still under discussion. Previously, we could not find a significant role for mucosal MT on Cd absorption by using an in situ technique (Kello et al 1979). One reason why we could not get a significant result may have been because the interval between the Cd perfusion and assay was very short. In order to clarify the role of MT induced by oral pretreatment with Cd or Zn on the absorption of Cd from a subsequent Cd exposure, we estimated the Cd absorption at 24 hr after the oral intubation of Cd into the mouse in vivo.

MATERIALS AND METHODS

Specific-pathogen-free male mice (ICR, 6 weeks old) were kept at a constant room temperature (25±0.4 °C) and humidity (42±5%), and were maintained on a controlled 12 hr light-dark cycle. Food (type M from Oriental Yeast Co., LTD, Tokyo, Japan) and water were provided ad libitum. After a one-week acclimatization period they were randomly assigned to one of the treatment groups.

Mice were deprived of food 12 hr before the intubation of metal or the sacrifice. In addition, food was given from 3 hr after the treatment with metal. The solutions (0.9% NaCl) of metal compounds ($CdCl_2$ or $ZnCl_2$) were

given into the lower part of the stomach with single doses of 50 μl-60 μl volume through polyethylene tubing (o.d. 1.0 mm).

The control group was orally given 50 μl of 0.9% NaCl solution 24 hr before the sacrifice. In order to preinduce the same content of mucosal MT (Ouellette et al 1982), the Cd-Cd and Zn-Cd groups were pretreated with Cd (a dose level of 7.5 mg/kg body weight) and Zn (a dose level of 30.0 mg/kg body weight), respectively. Twenty-four hr later, they received a large dose of Cd (22.5 mg/kg) and, 24 hr after that, were sacrificed. For comparison with these groups, one Cd group which was neither pretreated with Cd nor Zn was intubated with a large dose of Cd and 24 hr later killed. To estimate the pretreatment effect of Cd, two Cd-pretreated groups without a large dose of Cd were intubated with Cd (7.5 mg/kg), and 24 and 48 hr later killed. One Zn-pretreated group was used for estimating the intestinal Zn-MT stimulated by the pretreatment with Zn (30.0 mg/kg) alone. The group was killed at 24 hr after the intubation of Zn solution.

The proximal intestine (about 10 cm), liver and kidney were removed from the body. The intestine was cut longitudinally. To remove gastric content, it was washed with chilled 0.9% NaCl solution and then was homogenized by 10 volumes of 0.25 M sucrose with a Polytron (Type PT 10-35) homogenizer at 4 °C. A part of the homogenate (1.0 ml) was used for the metal measurement. To get the supernatant fraction, the remainer was centrifuged at 35,000 g for 30 min at 4 °C. The MT in this fraction was assayed by the modified method (Sugawara and Sugawara 1982) of Onosaka et al (1978).

The treatments, including digestion and dilution of liver (0.49±0.02 g), kidneys and intestinal homogenate (1 ml), were carried out by the method described previously (Sugawara 1977). Cd concentration was determined with an atomic absorption spectrophotometer (Model 208, Hitachi Corp., Tokyo, Japan).

RESULTS AND DISCUSSION

When the large dose (22.5 mg/kg) of Cd was given orally to the mice pretreated with Cd or Zn, the Zn-Cd and Cd-Cd groups showed a significantly higher MT concentration than that of the Cd group which was not pretreated with Cd or Zn (Table 1). The increase may have been due to the pretreatment effect of Cd or Zn on the MT induction. However, this increase did not lead to significant retention of Cd in the intestine (Table 1). The mucosal Cd seems likely to be the remaining Cd

which was not transported at this time (Taguchi 1985).

The absorbed Cd content during 24 hr was estimated from its content in the liver and kidney. The calculation was based on the assumption that once most Cd is absorbed, it is deposited in the liver and kidney, and that little of this organic Cd is excreted for 24 hr. The Zn-Cd group showed a slightly, but not significantly, lower liver and kidneys (L+K) Cd content than the Cd group. In order to estimate the absorption of Cd in the Cd-Cd group, 2.4 μg (mean of the Cd-pretreated group 2) was subtracted from the content of each mouse in this group. The absolute content of absorbed Cd was thus calculated to be 3.54±1.9 μg. This content was clearly lower than that (8.37±4.6 μg) of the Cd group which was not pretreated with Cd or Zn. The pretreatments with Zn or Cd may prevent the absorption of subsequently intubated Cd under our conditions.

Table 1 Mucosal Metallothionein and Cd Concentration, and Hepatic and Renal Cd Content

Group		Intestine		Liver	Kidney	L+K
		[a]MT	[b]Cd	[c]Cd	[c]Cd	[c]Cd
Control	(5)	7.4±0.9	[d]-	[d]-	[d]-	[d]-
Cd-pre. 1	(5)	13.3±3.6	7.9±4.3	1.5±1.0	0.28±0.1	1.71±1.1
Cd-pre. 2	(5)	[d]-	3.8±1.9	2.1±0.8	0.33±0.1	2.40±0.9
Zn-pre.	(4)	10.1±2.7	-	-	-	-
Cd	(6)	10.8±1.3	9.2±3.2	7.5±3.9	0.78±0.3	8.37±4.3
Zn-Cd	(6)	13.6±2.4*	10.1±2.4	5.7±4.3	0.68±0.3	6.42±4.6
Cd-Cd	(5)	16.0±2.9**	9.1±1.6	5.3±1.7	0.61±0.2	5.94±1.9 [e](3.54±1.9)

Results are expressed as mean±SD. The number of animals is given within parentheses. [a]MT (metallothionein) in the intestinal supernatant fraction (35,000 g for 30 min) is expressed as Cd (μg/g wet tissue) bound to the MT. [b]Cd (μg/g wet tissue): concentration in the intestinal supernatant fraction. [c]Cd (μg): content in the liver and kidney. [d]Assay was not carried out. [e]The calculation is seen in the text. Differences between Cd and, Zn-Cd and Cd-Cd groups were significant at *$p<0.05$ or **$p<0.01$ by the two-tailed Student's t-test.

In a related work, Kello et al (1979) reported previously that the mucosal MT induced by oral Cd did not function as a determinant on the absorption of the 109Cd perfused later (perfusion time 25 to 275 min). Recently, Folulkes and McMullen (1986) mentioned that 109Cd perfused in situ into the rat intestine in which Zn-MT was previously stimulated at several levels, was sequestered in the mucosa, and that it was not transported transmurally during the 40 min perfusion. It seems likely that the preinduced MT was a determinant in Cd absorption. The further fate of the 109Cd, which apparently was bound to the MT, was not elucidated in this report. Engstrom and Nordberg (1979) reported that the higher gastrointestinal absorption of 109Cd observed in mice pretreated with Cd was probably explainable by the known stimulatory effect of Cd exposure on the synthesis of intestinal MT. The differences among these results may be mainly due to the preinduced mucosal MT concentration and the procedure of Cd exposure.

In the proximal intestine of the Cd, Zn-Cd and Cd-Cd groups, tissue damage was not found macroscopically. We conducted a pilot study where Cd was intubated into mice at the level of 75 mg/kg. The mice showed severe mucosal impairments and their mortality rate increased. Squibb et al (1976) reported that when Cd was given orally at a level of 100 mg/kg, the uptake into the liver, kidney and testis was greater in rats pretreated with a small dose of Cd than in water-pretreated rats. Recently, however, Morita (1984) reported that a pretreatment with a small oral dose of Cd prevented the transport of a large dose of Cd (100 mg/kg) which was intubated later. The reasons proposed were different from each other, although the experimental procedures were almost identical. It appears that the challenged dose (100 mg/kg) used was too large to enable understanding of the mechanism of Cd absorption involving the role of MT.

Oral pretreatment of Cd or Zn stimulated MT induction, but did not increase the retention of Cd in the intestine. By pretreatment with Zn or Cd, the transport of Cd intubated subsequently was blocked for 24 hr. However, it does not necessarily follow that the inhibition of Cd transport was due to the MT function sequestering Cd. The enhancement of Cd uptake into the intestinal mucosa, which was probably found in the early phase, may not result in the eventual increased absorption of the metal from the gastrointestine.

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