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Metabolism of Orally Administered Cadmium-Metallothionein in Mice

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The main source of cadmium in the diet is cereal or meat, especially in liver and kidney. Since the cadmium in both liver and kidney is bound to metallothionein, a heat-stable protein, the gastrointestinal absorption and metabolism of cadmium metallothionein (CdMt) was studied in detail. The selective renal cadmium deposition after oral CdMt was analogous to the studies on injected CdMt. Metallothionein with ^{109}Cd or ^{35}S-cysteine radioactive label was isolated from rat liver and administered orally (60 μg Cd) through a gastric tube to mice (C57 BL/6J). After 4 hr, a major portion of the ingested CdMt was isolated intact from intestinal mucosal cells. However, only a small amount of cadmium bound metallothionein was present in the kidney supernatant. The protein moeity was also degraded completely in kidney. The absorption and tissue distribution of cadmium from oral cadmium-cysteine and cadmium-glutathione complexes were similar to that after oral $CdCl_2$ in mice. These results suggest that oral CdMt may be absorbed intact from the gastrointestinal tract and the protein is degraded during renal deposition.

Introduction

The critical organ in chronic cadmium toxicity is considered to be kidney; but the various factors influencing the movement and deposition of cadmium in the kidney are not yet understood clearly. Studies (*1–3*) on the metabolism of injected cadmium-metallothionein (CdMt) have shown that biocomplexes of cadmium may have a significant role in the tissue distribution and toxicity of cadmium. However, there is little information available regarding the gastrointestinal absorption of different dietary forms of cadmium and their toxicity. The average gastrointestinal absorption of cadmium from food is estimated to be about 6% of the total daily intake in humans (*4*). Studies (*5*) in human volunteers using radioactive cadmium salts showed that the absorption of cadmium was dependent on the iron status of the individual and was increased in iron deficiency. The deposition of cadmium in the kidney was also increased in iron deficiency in experimental animals (*5*).

It is now well known that cadmium is present as a heat-stable protein, metallothionein, in beef liver and kidney. In view of the potential toxic effects of injected CdMt on kidney (*2*) and its cellular toxicity on intestine in direct perfusion experiments (*6*), the absorption and tissue distribution of oral CdMt were studied in detail in the present investigation. In a preliminary study (*7*) from our laboratory comparing the gastrointestinal absorption of oral cadmium salts and CdMt, a similar absorption of cadmium from these two forms was observed. However, cadmium from oral cadmium salts was preferentially accumulated in the liver, whereas, the major depot for ingested CdMt was the kidney. Similar tissue distribution of cadmium was observed after CdMt injection (*1, 2*). The present study was carried out using radioactively labeled metallothionein in both metal and protein moiety to determine whether orally administered CdMt can reach the small intestine intact and can be absorbed without degradation.

Materials and Methods

Cadmium-metallothionein (CdMt) was isolated from livers of rats injected with 0.6 mg Cd/kg as cadmium chloride ($CdCl_2$) and either $^{109}CdCl_2$ or ^{35}S-cysteine. The method of isolation of ^{109}CdMt and ^{35}S-CdMt was the same as that described previously (*1, 8*). The final purification of metallothionein was achieved by chromatography on DEAE Sephadex A-25 columns (*9*) and the major metallothionein fraction (DEAE II fraction) was used in the present study. The purity of the isolated ^{109}CdMt and ^{35}S-CdMt was confirmed by their elu-

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tion as a single peak with ten thousand molecular weight in calibrated Sephadex G-75 columns. The cadmium content of these fractions was estimated by the flameless atomic absorption method (7) and the specific radioactivity of the isolated ^{109}CdMt was calculated. A solution of $^{109}CdCl_2$ with identical specific activity was prepared. ^{109}Cd-cysteine and ^{109}Cd-glutathione complexes were prepared by adding a four times excess molar concentration of either cysteine or glutathione to $^{109}CdCl_2$ solution and adjusting to pH 8.

Male mice of C57 BL/6J strain, 6-10 weeks old, were obtained from Jackson laboratory. They were fed with a commercial stock diet (Purina mouse chow from Ralston Purina Co.). The mice were starved overnight and one of the following cadmium solutions containing 60 μg of cadmium and 2 μci ^{109}Cd radioactivity in 0.2 ml was administered orally through a stomach tube under mild ether anesthesia: $^{109}CdCl_2$, ^{109}Cd-cysteine, ^{109}Cd-glutathione, ^{109}CdMt, and ^{35}S-CdMt. All the mice were sacrificed 4 hr after oral cadmium administration, and ^{109}Cd radioactivity was measured in liver, kidney, spleen, pancreas, testes, heart, and lung in all groups of mice except the one group given ^{35}S-CdMt. The total amount of radioactive cadmium deposited in organs was calculated. The intestinal contents were washed out with cold normal saline, the proximal one-half of the small intestine removed, the mucosa was scraped off with a glass microscopic slide and pooled in each group. The kidneys from ^{109}CdMt and ^{35}S-CdMt fed mice and the intestinal mucosa from all the different groups were homogenized in 0.25*M* sucrose, Tris-HCl buffer, pH 8.6. The tissue homogenates were centrifuged at 105,000*g* for 1 hr, and the postmicrosomal supernatants were fractionated on calibrated Sephadex G-75 columns for separation of proteins. The ^{109}Cd or ^{35}S radioactivity of Sephadex fractions were measured in a gamma or a liquid scintillation counter with counting efficiencies of 45% and 90%, respectively.

Results

Figure 1 shows the Sephadex G-75 filtration patterns of postmicrosomal supernatant fractions of kidney and intestine from mice, 4 hr after oral ^{109}CdMt and ^{35}S-CdMt. Even though most of the absorbed radioactive cadmium from oral CdMt was deposited in the kidney, a major portion of cadmium in the kidney supernatant was bound to high molecular weight proteins and not to metallothionein. There was also little ^{35}S-cysteine radioactivity associated with the renal metallothionein suggesting considerable degradation during the initial period of deposition in the kidney. In contrast to the renal

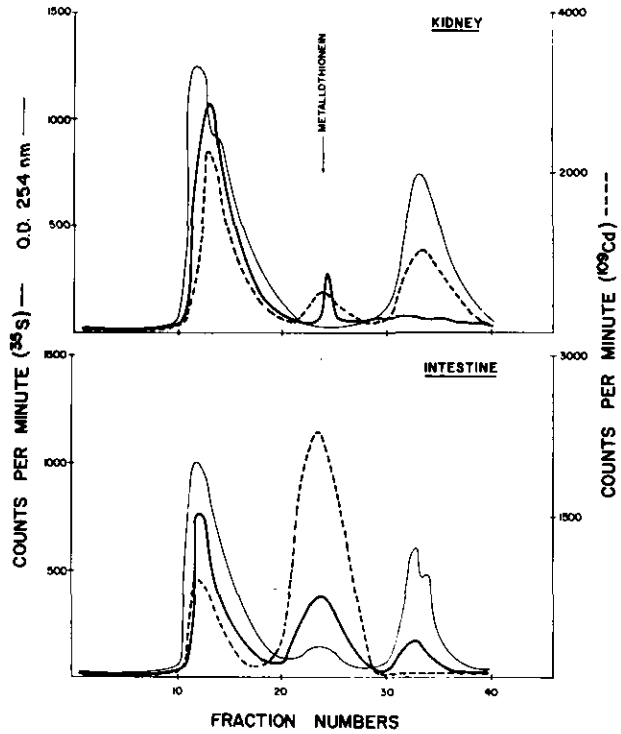

FIGURE 1. Gel filtration of kidney (upper panel) and intestine (lower panel) cytosols, 4 hr after oral administration of ^{109}CdMt or ^{35}S-CdMt in mice: (--) ^{109}Cd radioactivity; (——) ^{35}S radioactivity; (——) optical density at 254 nm. Cytosol samples were fractionated on calibrated Sephadex G-75 columns (0.9 × 60 cm). The arrow indicates the position of elution of metallothionein.

deposition, a major portion of the ingested metallothionein was isolated intact from the intestinal mucosa and a considerable amount of both ^{35}S-cysteine and ^{109}Cd radioactivity was associated with the metallothionein fraction. These results suggest that at least a portion of orally administered CdMt can reach the small intestine intact and can be reabsorbed without considerable degradation.

The tissue distributions of cadmium, 4 hr after a single oral dose of equal amounts of cadmium in the form of $^{109}CdCl_2$, ^{109}Cd-cysteine, ^{109}Cd-glutathione, and ^{109}CdMt in mice are compared in Table 1. Except for CdMt-fed mice, a major portion of absorbed cadmium was deposited in the liver in all other groups. The oral administration of CdMt in mice resulted in an increased deposition of cadmium in the kidney. The similar organ distribution of cadmium from oral $CdCl_2$, Cd-cysteine, and Cd-glutathione suggested similar gastrointestinal absorption of cadmium from cadmium salts and these biocomplexes.

The fractionation of the intestinal supernatant samples from mice, 4 hr after oral $CdCl_2$, Cd-cysteine, Cd-glutathione, and CdMt on Sephadex

Table 1. Tissue distribution of cadmium after oral administration of different biocomplexes of cadmium.[a]

	Cadmium in organ, ng			
	$CdCl_2$	Cd-Cysteine	Cd-GSH	CdMt
Liver	257.7 ± 32	214.3 ± 35	255.8 ± 41	18.1 ± 0.4
Kidney	30.5 ± 3	37.0 ± 6	33.8 ± 3	130.7 ± 0.8
Spleen	6.2 ± 0.9	2.6 ± 1.4	2.7 ± 1	2.2 ± 0
Pancreas	9.9 ± 4.3	14.6 ± 4.5	13.1 ± 6	5.5 ± 2
Testes	4.2 ± 0	3.1 ± 0.7	3.2 ± 0.9	2.0 ± 0
Heart	7.1 ± 1.5	5.4 ± 0.9	4.1 ± 0.1	0.9 ± 0.6
Lung	5.5 ± 0.5	5.6 ± 3.2	3.9 ± 0.1	4.3 ± 0.1

[a] Male mice (C57B1/6J) were starved overnight and 60 μg of Cd and 2 μCi of ^{109}Cd were administered as $CdCl_2$ or complexed with cysteine (2 μmole) and glutathione (2 μmole) or as rat liver cadmium-metallothionein (CdMt) orally with a gastric tube. They were sacrificed 4 hr later. The results are from four mice in each group.

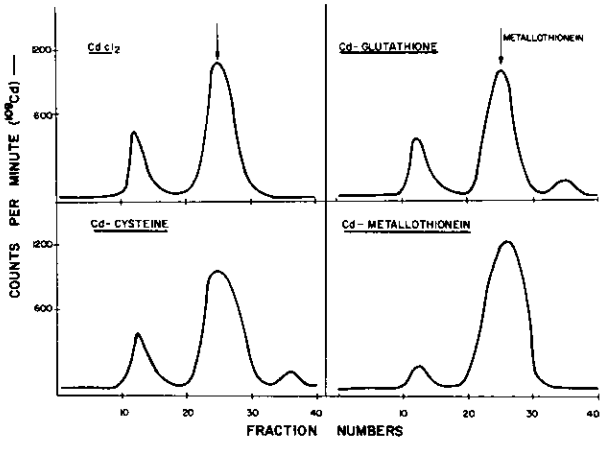

FIGURE 2. Distribution of ^{109}Cd in mice intestinal cytosols after a single oral administration of ^{109}Cd as $CdCl_2$ or Cd-cysteine or Cd-glutathione or Cd-metallothionein. The mice were sacrificed 4 hr after ingestion, the proximal half or small intestine was perfused, and the mucosal cell cytosols were fractionated on Sephadex G-75 columns.

G-75 columns showed a similar cadmium binding pattern (Fig. 2) in all the different groups. The major cadmium binding protein in the intestine was metallothionein. However, there was an increased amount of cadmium bound to metallothionein in the mucosal cells of CdMt fed mice as compared to groups fed $CdCl_2$, Cd-cysteine, and Cd-glutathione.

Discussion

Increased renal accumulation of cadmium with age has been reported in both general human population (*10, 11*) and in workmen who were continuously exposed to excessive amounts of cadmium (*4, 12*). The typical feature of chronic cadmium intoxication is the renal damage affecting both the glomerular filtration and proximal tubular reabsorption (*13*). However, little is known about the metabolic and biochemical events which precede the renal toxic effects of cadmium. Investigations (*14, 15*) from our laboratory on the role of CdMt in the cadmium induced nephropathy indicated that there were at least two distinct phases in chronic cadmium exposure studies in rat. The first phase was an initial tolerant phase, where CdMt was within the renal and hepatic cells without any cell injury. The second phase was a toxic phase characterized by detectable amounts of CdMt in plasma and renal tubular damage. Recent studies (*2, 15*) also revealed that the injection of CdMt can bypass the initial tolerant phase. Thus, the renal damage in experimental cadmium toxicity may be directly correlated to the extracellular presence of CdMt (*14*).

The important factors to be considered in renal toxicity of cadmium are the movement of cadmium to the kidney and release of CdMt to the extracellular compartment. The fractionation of intestinal mucosal cells and separation of the cytosol by Sephadex gel filtration, 4 hr after oral administration of ^{109}CdMt and ^{35}S-CdMt, showed that a major portion of both ^{109}Cd and ^{35}S ingested radioactivities were associated with intact metallothionein (Fig. 1). These results on the recovery of intact radioactive CdMt from the mucosal cells after oral CdMt suggested that oral CdMt could reach the small intestine and was probably absorbed without much degradation. It is not clear yet whether ingested CdMt is completely protected from degradation with proteolytic enzymes and low pH environment of the gastric content. The cadmium-bound metallothionein was subsequently released to the general circulation and sequestered by the kidney, probably similar to that after parenteral administration (*2*). The mechanism of transfer of absorbed CdMt from mucosal cells to kidney is presently unknown. However, fractionation of the kidney supernatant 4 hr after oral administration of radioactive CdMt revealed considerable degradation of the protein and only trace amounts of intact CdMt could be isolated from the kidney. These results were contrary to that after parenteral administration of CdMt (*1*) and suggested that ingested CdMt though absorbed intact from the gut, was degraded considerably during transfer to the kidney or soon after renal deposition.

The possible degradation of oral CdMt in the gastrointestinal tract and its subsequent absorption as a cadmium–cysteine complex can be further ruled out because of the differences in tissue distribution of cadmium after oral CdMt and Cd-cysteine. Cadmium from oral Cd-cysteine was accumulated mainly in liver similar to that after $CdCl_2$ (Table 1). A major question which arises from these

studies is whether oral CdMt can be toxic to the intestine and kidney. Preliminary morphological data in our laboratory with high doses (60 μg Cd as oral CdMt) showed morphological changes both in intestine and kidney, one week after oral CdMt (unpublished data). However, more results using lower doses of oral CdMt are needed to have a definite conclusion on the potential toxicity of oral CdMt. It should be mentioned that since cadmium was not released from CdMt by heating, the dietary form of cadmium may not be altered by cooking and for those who consume beef liver and kidney preparations, the major dietary form of cadmium may be metallothionein.

The direct extrapolation of these results on single exposure studies in mice to dietary intake of cadmium in humans may be difficult because of the well known interactions between cadmium and essential metals like iron and calcium in the diet (*5, 16*). However, these studies have significant implications on the importance of different dietary forms of cadmium, especially metallothionein in the gastrointestinal absorption and renal deposition of cadmium.

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