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Intracellular Metabolism and Effects of Circulating Cadmium-Metallothionein in the Kidney

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The mechanism of cadmium-metallothionein (CdMT)-mediated nephrotoxicity is being studied in rats using an acute dose regimen. Results of metabolism studies have shown that injected CdMT is rapidly degraded by the kidney with the release of Cd^{2+} into the cell cytoplasm. Ultrastructural studies indicate that an increase in the number of small lysosomes is the first measurable effect of CdMT in the kidney at 1 hr. This is followed by an increase in the number of small vesicles at 4 hr. It is proposed that these effects are the result of decreased primary lysosome formation and an inhibition of the fusion of pinocytotic vesicles with cell lysosomes by Cd. Functional alterations measured 8 hr after CdMT injection include an increase in urine volume and increased excretion of the low molecular weight protein, RNAase. Prior induction of renal MT by Zn pretreatment prevents the induction of polyuria and low molecular weight proteinuria by CdMT. These data provide further evidence that CdMT nephrotoxicity occurs as a result of Cd^{2+} toxicity within the cell.

The type of renal damage that is a primary consequence of chronic cadmium exposure is characterized by a low molecular weight proteinuria that is indicative of proximal tubule cell dysfunction (*1,2*). The mechanism by which renal toxicity develops during cadmium intoxication is unclear, but accumulating evidence suggests that cadmium-metallothionein (CdMT) might play a role. Recent data suggest that Cd is transported directly to renal proximal tubule cells in the form of CdMT (*3,4*) and studies with injected CdMT have shown that this small cadmium-containing protein induces a renal damage similar to ionic Cd by specifically affecting proximal tubule cells (*5–7*). On a Cd basis, the LD_{50} of CdMT is about 7 times that of $CdCl_2$ (*8*), due most likely to its preferential accumulation in the kidney (*5,6,9*).

Investigations are in progress to determine the mechanism of CdMT toxicity. In this regard there are currently two mechanisms under consideration. One proposes that CdMT damages the proximal tubule cell membrane in the process of its reabsorption from the tubular lumen (*6,10*). The second suggests that the toxic species is the Cd^{2+} released from the protein moiety following degradation of the protein within the proximal tubule cell (*8,11,12*). In this paper the metabolism and toxicity of CdMT are reviewed, and evidence is presented to support the hypothesis that CdMT toxicity is mediated via release of toxic levels of Cd^{2+} within the cell.

Renal Metabolism of Circulating CdMT

Circulating CdMT passes through the glomerulus and is reabsorbed by renal proximal tubule cells (*13*). Studies using ^{109}Cd and ^{3}H- or ^{35}S-labeled CdMT have shown that the protein is rapidly degraded by lysosomes and the Cd is released into the cell cytoplasm (*5,8,12*). Cain and Holt (*12*) reported that an intravenous dose of ^{3}H-CdMT is already partially degraded within 30 min of injection and degradation of the protein is virtually complete by 4 hr. Cytoplasmic Cd is eventually bound to newly synthesized renal metallothionein; however, at early time periods after injection, a significant amount of Cd is non-thio-

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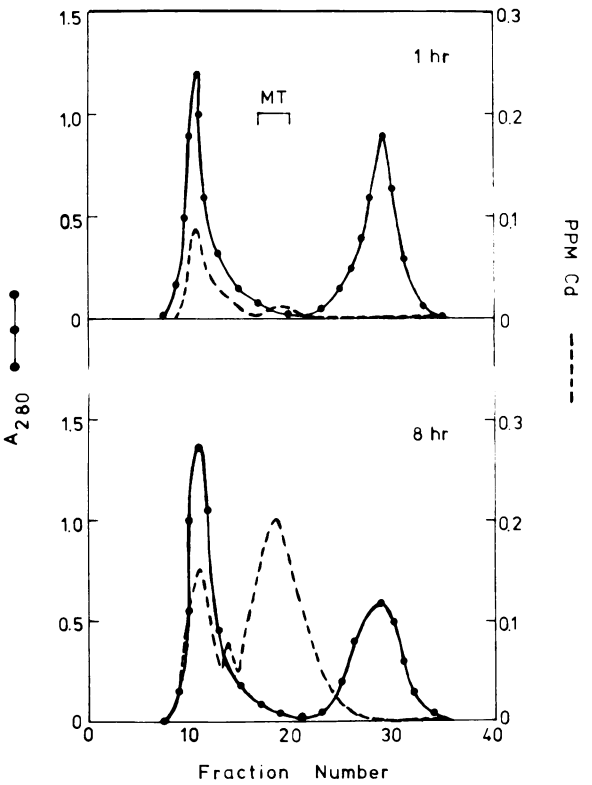

FIGURE 1. Sephadex G-75 chromatography of renal cytosol fractions from rats injected with CdMT (0.6 mg Cd/kg) 1 or 8 hr prior to sacrifice. Cd eluting with the void volume fraction is referred to as high molecular weight (HMW) protein-bound Cd. Approximately 70% of the Cd in the 8 hr sample is present as CdMT.

nein-bound (*5,11,14,15*). This is illustrated by the data shown in Figure 1, in which Cd is bound mainly to high molecular weight proteins at 1 hr after injection of a 0.6 mg Cd/kg dose of CdMT, however, by 8 hr approximately 70% of the cytoplasmic Cd is MT-bound. This suggests that the rate at which Cd is initially released into the cell is greater than the rate of new metallothionein synthesis by the kidney cells. This is consistent with data of Cain and Holt (*12*), indicating that MT synthesis is not initiated until between 2 and 4 hr after injection of CdMT.

Renal Toxicity of Circulating CdMT

Functional alterations induced by CdMT are, in part, dose-dependent. Nordberg and co-workers (*7*) have reported oliguria in mice 2–3 days after injection with CdMT at a dose of 1.5 mg Cd/kg. This dose resulted in 40% fatalities in 4 days. In rats injected with a nonlethal dose of CdMT (0.6 mg Cd/kg), however, urine volume was increased within 8 hr of injection and remained elevated for 5 days (Squibb et al., unpublished data). The difference in these responses is probably due to the different degree of renal failure and tubular blockage induced by the CdMT dose.

In addition to polyuria, a 0.6 mg Cd/kg dose of CdMT in rats induces low molecular weight proteinuria as evidenced by increased RNAase excretion (Fig. 2) and SDS gel electrophoretic analysis of urinary proteins (Squibb et al., unpublished data). Nordberg (*16*) has also reported the presence of tubular proteinuria in CdMT-treated mice.

Ultrastructural studies have shown that injected CdMT selectively affects renal proximal tubule cells. Glomeruli and distal tubules appear normal while proximal tubule cells are vacuolized and show signs of cell degeneration and desquamation into the tubular lumen over a 24 to 48 hr period (*5–7*). Morphometric analysis of early ultrastructural changes has shown that the number of small lysosomes in proximal tubule cells

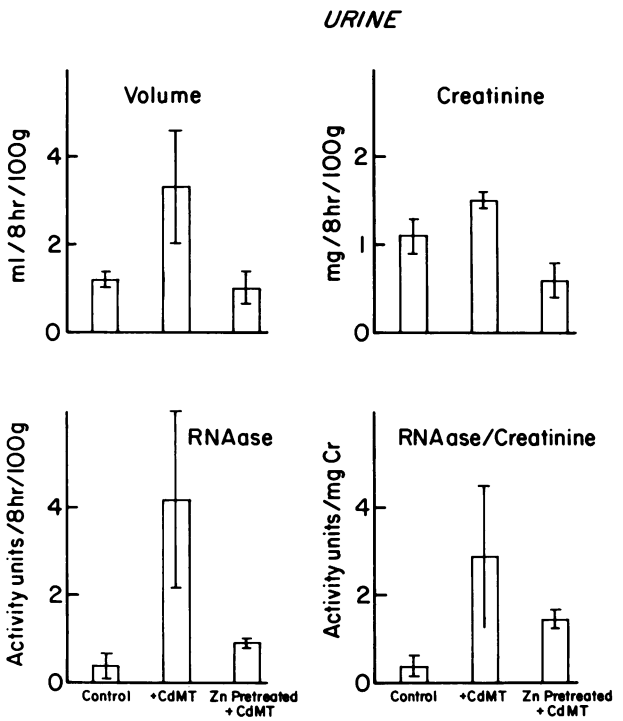

FIGURE 2. Effect of Zn pretreatment on the renal toxicity of CdMT. Rats were injected with 20 mg Zn/kg as $ZnSO_4$ (IP) 16 hr prior to injection with 0.6 mg Cd/kg as CdMT (IP). Controls were injected with saline. Urine was collected from the time of CdMT injection to the time of sacrifice (8 hr). Creatinine and RNAase activity were measured according to the procedures of Fabiny and Ertingshausen (*24*) and Imrie and Hutchison (*25*), respectively.

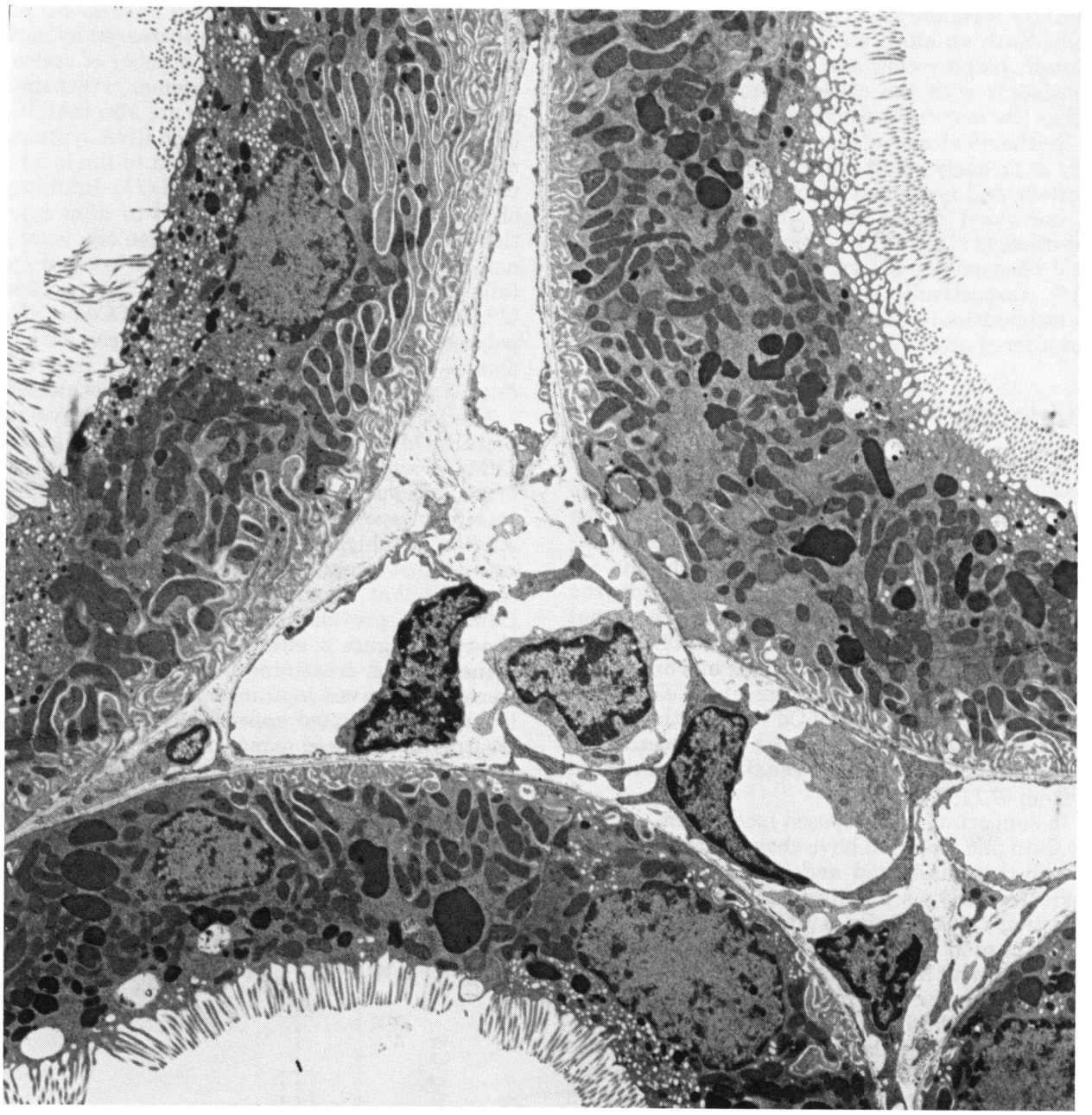

FIGURE 3. Electron micrograph of renal proximal tubule cells of a rat injected with 0.6 mg Cd/kg as CdMT 4 hr prior to sacrifice. Many of the cells contain an increased number of small apical vesicles and small lysosomes.

increases significantly within 1 hr of injection of a 0.6 mg Cd/kg dose of CdMT and almost triples by 4 hr. In addition, the number of small vesicles is increased dramatically at 4 and 8 hr (*11*). These changes are illustrated in the electron micrograph in Figure 3. At 4 hr after injection of CdMT, increased numbers of small vesicles and lysosomes are apparent in the apical portion of many renal proximal tubule cells. The mechanism by which CdMT produces these early ultrastructural alterations has not been clearly identified; however, evidence from our laboratory suggests that they might develop as a result of an inhibition of primary lysosome formation and decreased fusion of lysosomes with pinocytotic vesicles (Squibb et al., unpublished data). A similar cellular vesiculation occurs in maleate nephrotoxicity, in which transport of protein from endocytic vesicles to lysosomes is inhibited (*17*). Also, Mego and Cain (*18*) and Lillevold (*19*) have reported that Cd^{2+}

inhibits secondary lysosome formation in liver cells. Such an effect on the proximal tubule cell protein reabsorption and degradation system is consistent with the observation that CdMT induces low molecular weight proteinuria.

Biochemical alterations that occur in the kidney as an early result of CdMT treatment include nuclear and lysosomal reactions. RNA synthesis is decreased dramatically (89%) at 4 hr after injection (*11*), while lysosomal cathepsin D and acid phosphatase activity are inhibited 50% and 31%, respectively (*20*). Protein synthesis and mitochondrial respiration, on the other hand, are not altered after 4 hr (*11*).

Evidence for the Involvement of Cd^{2+} in CdMT Nephrotoxicity

Several lines of investigation suggest that the nephrotoxicity of CdMT is brought about by the release of Cd^{2+} within the cell following degradation of the protein moiety. In the chronic Cd exposure situation, when Cd feeds slowly into the kidney, toxicity does not occur until the renal metallothionein detoxification system (*21*) is saturated. In experimental situations in which acute doses of CdMT are injected, toxicity is exerted at much lower renal levels of Cd because the rate at which Cd is released into the cell exceeds the immediate capacity of the renal metallothionein system (*8,11,12*).

In support of this proposed mechanism, studies by Cain and Holt (*12*) have shown that soluble Cd is non-thionein-bound and therefore potentially toxic as early as 30 min after an intravenous injection of CdMT. By 4 hr, almost 70% of the renal burden of Cd is non-thionein-bound and, although new thionein synthesis is initiated between 2 and 4 hr after injection, even at 7 hr there is still a significant amount of non-thionein-bound Cd in the cells. It was not until 24 hr that most of the cellular Cd was recovered in the MT-bound form. These findings are consistent with data presented in Figure 1 which show that there is still a considerable amount of non-thionein-bound Cd present 8 hr after injection of a toxic (0.6 mg Cd/kg) dose of CdMT. These data indicate that there is a time span of at least 8 hr during which potentially toxic Cd is present in renal cells following injection of CdMT.

Many of the alterations induced by CdMT occur within the first 8 hr after injection, in keeping with the time interval during which Cd remains bound to non-thionein components. Functional alterations in urine volume control and protein reabsorption are detectable by 8 hr (Fig. 2). The number of small lysosomes is increased as early as 1 hr after injection and the number of vesicles is increased by 4 hr (*11*). Lysosomal cathepsin D and acid phosphatase activities are also inhibited by 4 hr (*20*). The effect of CdMT on RNA synthesis also appears to be directly related to the level of non-thionein-bound Cd in the cell (*11*). Inhibition of RNA synthesis is greatest at 4 hr after injection but is diminished by 8 hr when the level of non-thionein-bound Cd is lower due to renal metallothionein synthesis. Hidalgo and co-workers (*22,23*) have shown a similar effect of Cd on RNA polymerase activity in the liver. These authors demonstrated that the inhibition was inversely related to Cd binding to cellular metallothionein.

The fact that the presence of elevated levels of metallothionein in kidneys protects against CdMT toxicity also suggests that liberated Cd ions are the primary cause of CdMT toxicity. This was first reported by Webb and Etienne (*8*) in studies in which ZnMT pretreatment protected rats against lethal doses of CdMT. It has also been observed that functional alterations induced by CdMT are prevented by Zn pretreatment. As shown in Figure 2, effects of CdMT treatment on urine volume, creatinine and RNAase excretion were not observed in animals in which renal MT levels were elevated approximately 4-fold by Zn treatment. In these same animals, Zn pretreatment increased the binding of Cd to MT and decreased the non-thionein-bound Cd (Fig. 4). At 8 hr after CdMT treatment, the amount of Cd bound to the high molecular weight (HMW) proteins of kidney cytosol, as measured by Sephadex G75 chromatography, was decreased from 15.5%

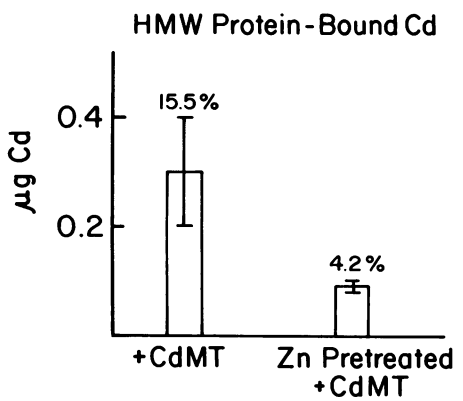

FIGURE 4. Effect of Zn pretreatment on the binding of Cd to high molecular weight (HMW) proteins 8 hr after CdMT (0.6 mg Cd/kg) treatment. HMW protein-bound Cd is the Cd present in the void volume fractions following Sephadex G-75 chromatography of kidney cytosol (see Fig. 1). Bars represent SDs; $n = 3$.

of the cytosolic Cd to 4.2% by Zn pretreatment. These data suggest that the elevated MT levels decrease the toxicity of CdMT by decreasing the non-thionein-bound pool of Cd and thus provide further evidence that Cd^{2+} released from injected CdMT is responsible for many of the toxic effects of CdMT.

In summary, the renal toxicity of CdMT is due to its rapid uptake and degradation by renal proximal tubule cells. Effects on lysosomal enzyme activities, RNA synthesis and possibly membrane fusion processes appear to be due to the rapid release of toxic Cd^{2+} ions within the cells.

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