Metallothionein-I/II Null Mice Are Sensitive to Chronic Oral Cadmium-Induced Nephrotoxicity

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Chronic exposure to cadmium (Cd) via food and drinking water is a major human health concern. We have previously shown that metallothionein (MT), a metal-binding protein, plays an important role in protecting against Cd toxicity produced by repeated sc injections. However, it is unclear whether MT protects against Cd-induced nephrotoxicity following chronic oral exposure, a route with obvious human relevance. To clarify this issue, MT-I/II knockout (MT-null) and background-matched wild-type (WT) mice were allowed free access to drinking water containing CdCl₂ (30, 100, and 300 ppm Cd), or feed containing CdCl₂ (100 ppm Cd) for 6 months, and the resultant nephrotoxicity was examined. Chronic oral Cd exposure produced a dose-dependent accumulation of Cd in liver and kidney of WT mice, reaching levels up to 50 μg Cd/g tissue. Immunohistological localization of renal MT indicated that chronic oral Cd exposure in WT mice greatly increased MT in the proximal tubules and the medulla, with cellular localization in both the cytoplasm and nuclei. As expected, no MT was detected in kidneys of MT-null mice. After 6 months of Cd exposure, tissue Cd concentrations in MT-null mice were only about one-fifth of that in WT mice. Even though the renal Cd concentrations were much lower in the MT-null mice, they were more sensitive than WT mice to Cd-induced renal injury, as evidenced by more severe nephropathic lesions, increased urinary excretion of γ-glutamyl-transferase and glucose, and elevated blood urea nitrogen. Six months of Cd exposure to MT-null animals resulted in greater increases in renal caspase-3 activity, an indicator of apoptosis, than to WT mice. In conclusion, this study demonstrates that lack of MT renders MT-null mice vulnerable to Cd-induced nephrotoxicity after chronic oral exposure, the primary route of human Cd exposure.

Key Words: cadmium; chronic oral exposure; nephrotoxicity, metallothionein-I/II null mice; histopathology; apoptosis; immunohistochemistry.

Cadmium (Cd) is an important inorganic toxicant widely distributed in the environment. This heavy metal is non-biodegradable, and the environmental levels of Cd are increasing as a result of industrial practices, as well as a contaminant of fertilizers (Goering et al., 1995; Jarup et al., 1998). Cd toxicity is very dependent on the dose, duration, and route of exposure. Acute Cd administration produces hepatic injury, pulmonary edema, and testicular damage, while chronic exposure results in renal dysfunction, osteomalacia, and cancer in multiple organs (Bhattacharyya et al., 1995; Friberg et al., 1986; Goering et al., 1995; Waalkes and Misra, 1996).

In humans, Cd exposure occurs primarily through ingestion and inhalation. Plants readily take up Cd, and the major route of exposure for the general population is via food, particularly Cd-contaminated rice, vegetables, and shellfish (Friberg et al., 1986). Tobacco use is also a significant source of Cd exposure. Renal tubular damage is probably the most common adverse effect of Cd exposure in humans, both in the general population and in occupationally exposed individuals (Goyer and Cherian, 1995). Because of its long biological half-life, tissue-Cd levels generally increase with age (Friberg et al., 1986), and kidney is the major site of Cd accumulation (Goyer and Cherian, 1995). In fact, present-day human exposure to Cd results in renal Cd concentrations that have been shown to produce kidney injury in approximately 7% of the general population (Jarup et al., 1998). There is also evidence that Cd exposure may be associated with renal neoplasia in humans and rodents (Kolonel, 1976; Mandel et al., 1995; Waalkes et al., 1999).

Most of the total body burden of Cd in animals and humans is associated with metallothionein (MT) (Goering et al., 1995). MT is a small protein, and one-third of its amino acid residues are cysteines. These cysteine residues bind and store metal ions, and play an important role in Cd detoxication (Klaassen et al., 1999). We have previously shown that MT-null mice are approximately 10 times more susceptible than WT mice to Cd toxicity following repeated sc injections, indicating the importance of MT in protecting against Cd toxicity. However, because food is the main source of Cd exposure for the general human population, the effects observed following repeated subcutaneous (sc) injections of Cd in animals might not accurately model oral exposures in humans. Furthermore, there are contradictory reports in regard to the inhibitory role of MT in
Cd absorption from the intestine (Lehman and Klaassen, 1986; Liu and Klaassen, 1996; Min et al., 1991; Rajan et al., 1999). It is also known that the CdMT complex, when isolated and injected into animals, is highly nephrotoxic, indicating that MT may play a key role in the causation of Cd nephropathy. The precise role of MT in either prevention or causation of chronic Cd-induced renal toxicity after oral exposure has not been established. Therefore, this study was designed to determine the toxic effects of Cd after oral exposure, and to determine whether intracellular MT also protects against oral Cd toxicity, as it does after parenteral exposure. To define the role of MT in Cd toxicity, MT-I/II knockout (MT-null) mice (Masters et al., 1994) were compared to wild-type (WT) mice given Cd in their water or feed. This study provides convincing evidence that intracellular MT is important in protecting against Cd-induced nephrotoxicity via oral exposure.

MATERIALS AND METHODS

Chemicals. CdCl₂ was obtained from Fisher Scientific Co. (Fair Lawn, NJ), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GT), creatinine, glucose, and blood urea nitrogen (BUN) kits were obtained from Sigma Chemical Co. (St. Louis, MO). N-acetyl-β-D-glucosaminidase (NAG) kits were purchased from Boehringer (Indianapolis, IN). All other chemicals were of reagent grade.

Animals. Homozygous MT-I and -II knock-out (MT-null) mice (129-Mt₁tm₁Bri Mt₂tm₁Bri, 129/SvPCJ background, Masters et al., 1994) were obtained from Jackson Laboratories (Bar Harbor, ME). The homozygous mutants were mated inter se to maintain the line. Genetic background-matched (129/SvImJ) wild-type (WT) mice and MT-null mice were bred in the AAAALAC-accredited facility at University of Kansas Medical Center. Mice were housed 4 per cage with a 12-h light/dark cycle at 22 ± 1°C. For control groups, male and female mice were allowed free access to mouse chow (PMI Nutrition International, Inc. 5015, Brentwood, MO) and tap water. For treatment groups, male and female mice (4/sex/group) aged 6–8 weeks were allowed free access to feed containing CdCl₂ (100 ppm Cd, prepared by Purina TestDiet, Richmond, IN), or 30, 100, or 300 ppm Cd as CdCl₂, in drinking water for 6 months. The mice were observed daily and body weights were recorded weekly to determine the effect of Cd on the general health of the animals. At the end of the study animals were weighed, euthanized with CO₂, and necropsied.

Urinary analysis. At the end of the experiment, mice were hydrated by gastric intubation (50 ml/kg, po), and then placed in metabolic cages to collect urine for a 24-h period (Liu et al., 1998b). Urine samples were analyzed for creatinine, glucose, protein, γ-GT, and NAG. Protein concentration in urine was determined by a dye-binding method, with bovine serum albumin as the standard (Bradford, 1976). Urinary glucose concentration was measured by the hexokinase/glucose-6-phosphate dehydrogenase method, creatinine concentration was determined by the Jaffe reaction, and γ-GT by the release of 5-amino-2-nitrobenzoate from the substrate γ-glutamyl-3-carboxy-4-nitroanilide, using commercially available kits from Sigma. N-acetyl-β-D-glucosaminidase was measured colorimetrically using a Boehringer reagent kit (Indianapolis, IN). The urinary creatinine concentration was used to normalize all other urinary parameters.

Tissue and blood analysis. At the end of the experiment, animals were anesthetized and decapitated to collect blood, and livers and kidneys were removed and weighed. Half of one kidney was fixed in 10% neutral formalin, processed by standard histopathological techniques, and stained with hematoxylin and eosin for light microscopic examination. The other half kidney was digested in nitric acid, and Cd and Zn concentrations were assayed by atomic absorption spectrophotometry. The contralateral kidney was homogenized in 20 mM Tris-HCl (pH 7.4) containing 1% Triton-100 and 150 mM NaCl, centrifuged at 12,000 × g for 10 min, and the resulting supernatants were used for determining caspase-3 activity, using Ac-DEVD-AMC as substrate, and fluorescence detection at λex 400 nm and λem 500 nm (Gillardon et al., 1997). Serum was analyzed for BUN and ALT using commercially available kits from Sigma (St. Louis, MO).

For histopathological analysis, proximal tubule degeneration was considered to be present when the tubular epithelial cells showed cell swelling, the presence of clear vacuoles of various sizes without peripheral displacement of the nucleus (hydroptic degeneration), desquamation of the apical portion of the cell, and loss or simplification of cell structure, including the plasma membrane. We considered tubular degeneration to be severe when it is accompanied by dissolution of the nucleus (karyolysis) in the absence of features of necrosis. The tubules were considered to be atrophic when there was a significant decrease in cell size, especially in the height of the tubular epithelium, dilatation of the tubule, and, in some cases, complete disappearance of the tubules. Tubular cells were considered apoptotic when they showed shrinkage and increased density of the nucleus (pyknosis), fragmentation of the nucleus (karyorrhexis), shrinkage of the cell, and increased cytoplasmic eosinophilia. Identification of cells with these features within the cytoplasm of normal cells (phagocytosed by the normal cell) was considered further proof of apoptosis. Interstitial nephritis was diagnosed in the presence of local or diffuse accumulation of inflammatory cells in the interstitium. Interstitial fibrosis was defined as increased formation of fibrous tissue in the interstitium due to chronic inflammation. Glomerular sclerosis was defined as thickening of glomerular basement membrane, increased mesangial matrix, absence of inflammatory cells within the glomerulus, and progressive simplification of the structure (or loss of structure) of the glomerulus. The severity of these renal lesions was graded as we described previously (Liu et al., 1998a).

Immunocytochemical localization of MT in kidneys. Kidney sections were dewaxed in xylene and rehydrated with serial solutions of graded alcohol, and endogenous peroxidase was blocked with hydrogen peroxide in phosphate-buffered saline. The sections were then incubated with monoclonal antibody (E-9) against MT-I/II (1:100) (DAKO, Carpinteria, CA) at 4°C overnight, followed by incubation with rat anti-mouse biotinylated IgG secondary antibody (1:200) for one h, and the signals were visualized using an ABC immunostain kit from Santa Cruz Biotechnology (Santa Cruz, CA).

Statistics. Data are expressed as mean ± standard error of 8 mice. Comparisons between WT and MT-null mice at identical doses were performed by the Student’s t-test. Multiple comparisons between control and treated groups were performed by one-way analysis of variance (ANOVA), followed by Duncan’s multiple-range test. Significance was set in all cases at p < 0.05.

RESULTS

Chronic oral consumption of Cd in drinking water (30, 100, or 300 ppm Cd) or feed (100 ppm Cd) had no apparent effects on body-weight gain of either MT-null or WT animals (data not shown), and all animals survived the 6-month exposure period.

Enlarged kidneys are indicative of inflammatory and proliferative lesions following repeated sc Cd injections (Liu et al., 1998b). At the concentrations of Cd used in the present study, chronic oral exposure to Cd in drinking water or in feed did not affect the kidney/body weight ratios in WT mice except for 300 ppm Cd in water (Fig. 1). However, kidneys and livers of MT-null mice receiving high doses of Cd (300 ppm Cd in the drinking water or 100 ppm Cd in the feed) were enlarged, resulting in greater kidney/body weight and liver/body weight ratios compared to WT mice.
Chronic consumption of Cd in drinking water resulted in a dose-dependent accumulation of Cd in kidneys of WT mice (Fig. 2). In WT mice receiving 300 ppm Cd in water or 100 ppm Cd in feed, the kidneys accumulated approximately 50 μg Cd/g. In comparison, the renal Cd concentration in MT-null mice was less than 10 μg/g kidney (Fig. 2). Interestingly, renal zinc (Zn) concentrations in MT-null mice receiving Cd in feed (100 ppm) were actually lower than control levels. This is in contrast to the increased Zn in WT mice given Cd in the drinking water at 300 ppm.

Immunocytochemical localization of MT in kidneys of WT (Figs. 3A and 3C) and MT-null mice (Figs. 3B and 3D) was also performed. Constitutive expression of MT in the kidney is very low, and immunostaining for MT was barely visible (data not shown). However, following chronic Cd exposure, renal MT in WT mice can be increased as much as 100-fold (Liu et al., 1998b). Thus, strong immunostaining for MT in WT mice receiving oral Cd in the water (300 ppm) was observed. Cd-induced MT was located mainly in the proximal tubular cells (Fig. 3A). The positive staining was also detected in the renal medulla (Fig. 3C). MT was detected not only in the cytoplasm, but also in the nucleus of WT mouse kidney (Fig. 3A and 3C arrowheads). As expected, no immunostaining of MT was found in the kidney of the MT-null mouse (Figs. 3B and 3D).

Urinary excretion of glucose, protein, and enzymes are indicative of renal injury. Chronic consumption of Cd in water (30, 100, or 300 ppm) or in feed (100 ppm) did not increase the urinary excretion of γ-GT, NAG, glucose, or protein in WT mice. However, in MT-null mice, these dosages of Cd did increase the urinary excretion of γ-GT and glucose (Fig. 4). These dosages of Cd did not significantly increase urinary excretion of protein or NAG in MT-null mice (Data not shown).

Blood biochemistry, and in particular blood urea nitrogen (BUN) concentration, was also used as an indicator of renal dysfunction. BUN was not increased in WT mice after consuming the various concentrations of Cd in water or in feed for 6 months, but it was increased in MT-null mice receiving 300 ppm Cd in water, indicating renal injury had occurred in the MT-null mice (Fig. 5). No apparent alterations in serum ALT activity, an indicator of liver injury, were observed in either
WT or MT-null mice under the current experimental conditions (data not shown).

Figure 6 shows representative photomicrographs of kidneys from WT and MT-null mice that had received normal mouse chow and unaltered water (Figs. 6A and 6B), Cd in water (300 ppm) (6C and 6D), or Cd in feed (100 ppm; 6E and 6F) for 6 months. Normal renal morphology was observed in both WT (Fig. 6A) and MT-null (Fig. 6B) control mice. Addition of Cd to the drinking water resulted in proximal convoluted tubule degeneration (Fig. 6C, arrowheads) in WT mice. Similar lesions were present in MT-null mice, but with more severe and extensive proximal convoluted tubule degeneration (Fig. 6D, arrowheads). In addition, MT-null mice showed severe proximal convoluted tubule atrophy, chronic inflammation (Fig. 6D, arrows), interstitial fibrosis, and dilated collecting tubules. Mice exposed to Cd in the feed had glomerular swelling (Fig. 6E) and severe tubular degeneration in WT mice. In addition to these lesions, MT-null mice showed extensive necrosis (Fig. 6F, arrows), marked atrophy and cystic dilation of the proximal convoluted tubules, apoptosis (Fig. 6F, small arrowheads), and tubular degeneration (Fig. 6F, large arrowhead). Other renal lesions in MT-null mice fed Cd in feed included interstitial nephritis and fibrosis, tubular atrophy in medulla, dilated collecting tubules, and foci of microcalcification. Both treatments (Cd in water and Cd in feed) produced more severe and more frequent renal damage in all segments of the nephron, and induced active tubular regeneration in MT-null mice as compared to WT mice. Comparisons of Cd-induced renal lesions produced by exposure of WT and MT-null mice for six months to Cd in drinking water and in feed are presented in Table 1. MT-null mice show increased susceptibility to Cd-induced renal cortical and medullary injury compared to WT mice. Again, damage to the proximal convoluted tubules caused by Cd in water and in feed was particularly severe in MT-null mice.

The observed apoptotic lesions in the kidneys were quantified by assaying caspase-3 activity. Chronic consumption of Cd in feed increased caspase-3 activity in WT mice (20%), but much greater increases were observed in MT-null mice fed 100 ppm Cd in feed (300%), or 300 ppm Cd in water (200%). These findings, together with the morphological observations,
FIG. 6. Representative photomicrographs of kidney sections from control mice and mice treated with Cd in water or in feed for 6 months; original magnification 400×. (A) Normal kidney section from a control wild-type mouse. (B) Normal kidney section from a control MT-null mouse. (C) Kidney section from wild-type mouse treated with Cd, 300 ppm in water, showing mild degeneration of the proximal convoluted tubules (arrowheads). (D) Kidney section from MT-null mouse treated with Cd, 300 ppm in water, showing degeneration of proximal convoluted tubules (arrowheads) and chronic inflammation (arrows). (E) Kidney section from wild-type mouse treated with Cd, 100 ppm in feed, showing glomerular swelling and degeneration of proximal convoluted tubules (all proximal tubules in the section show some degree of degeneration). (F) Kidney section from MT-null mouse treated with Cd 100 ppm in feed, showing extensive necrosis of proximal convoluted tubules (areas with loss of cellular details; arrows) and some apoptosis (small arrowheads); tubular degeneration is also present (large arrowhead).
suggest that lack of MT rendered MT-null mice more vulnerable to apoptotic lesions from chronic Cd exposure (Fig. 7).

**DISCUSSION**

The present study demonstrates that chronic oral Cd exposure produces renal injury, particularly in MT-null mice, as evidenced by histopathological changes, increased urinary excretion of γ-GT and glucose, and elevated BUN. Similar to repeated sc injections of Cd (Liu et al., 1998b), MT-null mice were more sensitive to chronic oral Cd-induced nephrotoxicity, via either drinking water or feed. This establishes the role of MT as protective, rather than facilitatory, in the nephrotoxicity produced by oral Cd intake.

Oral intake is a primary concern in human Cd exposure, particularly of the general population, and present day exposure levels appear to carry a significant risk for renal damage (Jarup et al., 1998). It is well known that the toxic effects of Cd are dependent on the dose, route, and duration of Cd exposure. In general, Cd given via the oral route is much less toxic than when given parenterally, primarily because Cd is poorly absorbed from the gastrointestinal tract (Goon and Klaassen, 1989; Lehman and Klaassen, 1986; Liu and Klaassen, 1996). Only about 0.5–3% of ingested Cd is absorbed from the gastrointestinal tract (Liu and Klaassen, 1996). The intestinal absorption of Cd is also affected by many factors, and the role of intestinal MT in Cd absorption is an issue of debate. Intestinal MT has been hypothesized to limit intestinal Cd absorption (Min et al., 1991; Rajan et al., 1999). However, this inhibitory role of intestinal MT in blocking Cd absorption has not been confirmed in models where MT is either greatly overexpressed, using MT-transgenic mice (Liu and Klaassen, 1996) or induced by Zn (Goon and Klaassen, 1989), or MT is minimally expressed, using MT-null animals (Kimura et al., 1998). In the present study, Cd accumulation in the liver and kidney of the WT mouse was 5-fold greater than that observed in the MT-null mouse. The decreased Cd accumulation in tissues of MT-null mice, however, is probably due to increased Cd elimination (Liu et al., 1996a), because MT is a major factor for tissue Cd retention (Engstrom and Nordberg, 1979; Klaassen et al., 1999).

The precise role of MT in chronic Cd nephrotoxicity has been debated for decades. The CdMT complex is a very potent nephrotoxicant when isolated and injected into animals (Che-rian et al., 1976; Nordberg et al., 1975). In this regard, MT has been thought to play a paradoxical role in Cd-induced nephrotoxicity. First, it is beneficial for protection against Cd hepatotoxicity (Goering and Klaassen, 1984), but it is harmful to

**TABLE 1**

Comparisons of Renal Injury Produced by Exposure of Wild-type (WT) and MT-null Mice to Cd in Drinking Water (300 ppm/W) or in feed (100 ppm/F) for 6 Months

<table>
<thead>
<tr>
<th>Lesions</th>
<th>WT, Cd 300/W</th>
<th>MT-null, Cd 300/W</th>
<th>WT, Cd 100/F</th>
<th>MT-null, Cd 100/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT degeneration</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>PCT necrosis</td>
<td></td>
<td>+</td>
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<tr>
<td>PCT apoptosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>PCT atrophy</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regenerating tubules</td>
<td>±</td>
<td>+</td>
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<tr>
<td>Glomerular swelling</td>
<td>+</td>
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<td>Glomerular sclerosis</td>
<td></td>
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<tr>
<td>Tubular atrophy (medulla)</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>Interstitial nephritis</td>
<td>+</td>
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<tr>
<td>Interstitial fibrosis</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Dilated collecting tubules</td>
<td></td>
<td>+</td>
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<tr>
<td>Scattered foci of microcalcification</td>
<td>+</td>
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</table>

*Note.* PCT, proximal convoluted tubule; ±, minimal; +, mild; ++, moderate; ++++, severe.

**FIG. 7.** Effect of consumption of Cd in water (W; 0, 30, 100, and 300 ppm) or feed (F; 100 ppm) for 6 months on renal caspase-3 activity in wild-type (WT) and MT-null mice. Data are mean ± SE of 8 mice. Asterisk indicates significant difference from wild-type mice, *p < 0.05.* ′p < 0.05 vs. control WT mice, ″p < 0.05 vs. control MT-null mice.
the kidney when the CdMT complex is released into the general circulation (Chan et al., 1993; Dudley et al., 1985). However, MT-null mice, which cannot form the CdMT complex, actually show more severe nephrotoxicity than WT mice following repeated Cd injections, indicating that CdMT complex is not essential for Cd-induced renal injury to occur (Liu et al., 1998b). The acute injection of CdMT complex has been proposed as a model for Cd-induced nephropathy (Cherian et al., 1976; Nordberg et al., 1975). However, this model is questionable because: (1) acute CdMT injection does not mimic the pathological changes in the kidney that occur during chronic Cd exposure (Liu et al., 1998a), (2) MT-transgenic mice that overexpress renal MT are not protected from acute CdMT nephrotoxicity (Liu et al., 1996b), (3) MT-null mice are not more sensitive to acute CdMT-induced renal injury (Liu et al., 1996b), but are more sensitive to chronic CdMT nephropathy (Liu et al., 1999), and (4) acute nephropathy can be induced by Cd in association with complexes other than MT, such as Cd-cysteine (Min et al., 1986). Thus, the results of the present study using chronic oral Cd exposure further support the hypothesis that MT is protective for the kidney, in a fashion similar to that for the liver (Goering and Klaassen, 1984), and cast further doubt on the concept that the CdMT complex is essential for Cd-induced chronic nephrotoxicity.

There are several potential explanations for the exacerbation of chronic Cd-induced nephrotoxicity in MT-null mice. The current theory holds that Cd binds to MT, and the sequestration of Cd by MT in the cytosol renders it “toxicologically inert” (Klaassen et al., 1999). Although the total renal accumulation of Cd in MT-null mice was only one-fifth of that in WT mice, it is the non-MT-bound Cd that is likely to be responsible for the resultant tissue injury (Goyer et al., 1989). Thus, even though much less Cd accumulates in kidneys of MT-null mice, it has a greater toxic impact. This supports the concept that it is the free or non-MT-bound Cd that is the toxic species in the kidney, and the levels of non-MT-bound Cd appear more important than total Cd as the determinant for renal injury in chronic Cd exposure.

The sequestration of Cd by MT does not exclude other possible roles for MT in protecting against chronic Cd nephropathy. For instance, MT-null cells (Kondo et al., 1997) and animals (Deng et al., 1999; Liu et al., 1998c) have been shown to be more sensitive to apoptotic lesions produced by radiation and cisplatin. Apoptosis is a characteristic of chronic Cd-induced renal injury, either following exposure to CdCl₂ (Hamada et al., 1991; Liu et al., 1998b; Tanimoto et al., 1993), or after exposure to CdMT (Liu et al., 1999). Consistent with these prior findings, the present study showed that MT-null mice had more apoptotic lesions than WT mice following chronic oral Cd exposure, as evidenced by both histopathology and increased caspase-3 activity. Caspase-3, an enzyme critical to the dedication of cells to apoptosis (Cohen, 1997; Thornberry and Lazebnik, 1998), can be inhibited by zinc (Chai et al., 1999; Perry et al., 1997). In fact, it is suspected that zinc, particularly free zinc, may be an important physiological controlling factor in apoptosis (Chai et al., 1999). In the present study, the renal zinc concentration in the MT-null mice was significantly lower than in WT mice after chronic oral exposure to Cd in feed. Because zinc may be a controlling factor for apoptosis (Chai et al., 1999), this could potentially render renal cells of MT-null mice more sensitive to apoptotic stimuli, consistent with our data on increased caspase-3 activity in chronic Cd-treated MT-null animals.

The increased renal injury from chronic Cd exposure in MT-null mice could potentially result from increased oxidative stress. It has been proposed that oxidative damage is involved in chronic Cd nephropathy (Bagchi et al., 1997; Shaikh et al., 1999). MT, based on its sulphydryl-rich groups, has also been hypothesized to function as a free radical scavenger (Klaassen et al., 1999; Lazo et al., 1995; Sato and Bremner, 1993). Lack of MT would then compromise the antioxidant capacity in MT-null mice, resulting in their being more sensitive to oxidative damage. MT-null cells are indeed more sensitive to oxidant-induced damage than similarly derived wild-type cells (Lazo et al., 1995).

In WT mice, renal MT protein increased more than 100-fold after repeated sc Cd injections (Liu et al., 1998b), and more than 50-fold following oral exposure to Cd in feed for 4 months (Liu et al., 2000). As expected, in the present and previous studies no induction of MT protein is observed in kidneys of MT-null mice. However, little is known about the localization of renal MT. In the present study, immunohistochemistry was utilized to localize renal MT following chronic oral exposure to Cd. The increased renal MT protein was detected not only in the renal cortex, but also in the renal medulla in WT mice. No positive staining was detected in MT-null mice. In addition, MT was detected not only in the cytoplasm, but also in the nuclei of proximal tubular cells of WT mice. This is an important finding in that the renal MT localization is in good accordance with the target sites of Cd toxicity. In MT-null mice, renal lesions were observed throughout the nephron, suggesting that the increased nephrotoxicity in MT-null mice is closely related to the inability of these mice to synthesize MT throughout the kidney.

In conclusion, the present study confirms that chronic oral Cd administration, via either drinking water or feed, produces nephrotoxicity in mice. More importantly, MT-null mice are clearly more sensitive than WT mice to Cd-induced renal injury, supporting a role for MT in protecting against, rather than causing, Cd nephrotoxicity.

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