METALLOTHIONEIN TRANSGENIC AND KNOCK-OUT MOUSE MODELS IN THE STUDY OF CADMIUM TOXICITY

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ABSTRACT—The role of MT in Cd toxicology has become clearer by the use of MT-I transgenic and MT-I and -II knock-out animals. We have shown that:(1)MT-transgenic and -null mice have altered tissue MT protein levels;(2)MT-transgenic and -null mice appear to be normal in other detoxifying systems examined, except for slight alterations in tissue Zn concentration;(3)MT does not appear to inhibit Cd absorption from the gastrointestinal tract, nor affect Cd tissue distribution;(4)MT reduces the elimination of Cd from liver;(5)MT protects against acute inorganic Cd-induced lethality and hepatotoxicity, and the mechanism of the protection appears to be due to its ability to sequester Cd in the cytosol, thus reducing the amount of Cd in critical organelles;(6)MT modulates Cd-induced expression of protooncogene(c-jun) and tumor suppress genes(p53) in mouse liver;(7)MT does not protect against CdMT-induced acute renal injury, and Zn-induced protection against CdMT-induced acute nephrotoxicity does not appear to be mediated through MT;(8)Chronic Cd administration produces renal injury inb MT-null mice, indicating that Cd-induced nephrotoxicity is not necessarily mediated through the CdMT complex;(9)MT protects against chronic CdCl2 nephropathy, suggesting that intracellular MT is an important adaptive mechanism decreasing CdCl2 nephrotoxicity, and that a single injection of CdMT may not be a good model to study chronic Cd nephropathy;(10)genetic background of mouse strains, rather than constitutive MT levels, is a more important determinant for Cd-induced acute testicular injury.

In addition to Cd detoxication, MT-transgenic and MT-null mice are also good models to determine other functions of MT. MT plays important roles in maintaining Zn homeostasis and protection against Zn toxicity. Knock-out of the MT gene also renders animals/cells more vulnerable to oxidative stress and DNA alkylating agent-induced toxicity. Therefore, the MT-transgenic and knock-out mouse models provide complementary approaches to those used previously, and have greatly increased our understanding of the role of MT in Cd toxicology, as well as other biological functions of MT.

KEY WORDS: Cadmium, metallothionein, metallothionein transgenic and knockout animals, hepatotoxicity, nephrotoxicity

INTRODUCTION

Cadmium(Cd), an extremely toxic metal, is now recognized as an environmental hazard to the general population. Cd is widely used by industry in electroplating, batteries, plastic stabilizers, and pigments. Cd is also a by-product of zinc and lead mining and smelting. Cd is nonbiodegradable and industrial uses are causing environmental levels of Cd to increase [1]. Cd is toxic to a number of organs, including the pulmonary, cardiovascular, renal, hepatic, skeletal, and immune systems [2]. In humans, acute Cd inhalation results in pulmonary edema and respiratory tract irritation, while chronic exposure to sufficient Cd via any route is manifested by renal tubular dysfunction, osteomalacia, bone fractures and tumors [1-4].

Most of the total body burden of Cd in laboratory animals and humans is associated with metalloth-
ionein(MT), especially in liver and kidney. MT is a low-molecular-weight protein, ubiquitous in the animal kingdom. MT has an unusual amino acid composition, containing no aromatic amino acids, while one-third of its residues are cysteines. These cysteine residues bind and store metal ions [5]. Moreover, it is a small protein easily induced by heavy metals, hormones, acute stress, and a great variety of chemicals [5,6]. Four major isoforms of MT have been identified. MT-I and MT-II exist in all tissues examined [5], whereas MT-III is located only in brain [7], and MT-IV in stratified squamous epithelia [8]. The cysteine content and the capacity of these MT isoforms to bind Cd are similar.

Since MT was discovered in 1957, extensive research has been conducted to define its biological and toxicological functions [5]. Most studies aimed at determining MT functions have utilized various chemicals to increase tissue MT levels. However, the obvious confounding issue is that MT inducers also produce many other effects in addition to increasing MT concentrations [9]. Currently, the best approach to determine the function of a protein is to use genetically altered animals that overexpress(transgenic) or fail to express that protein(knock-out). MT-I transgenic mice

Fig. 1. Serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities in control and MT-transgenic (MT-TG) mice 24 hrs after injection of a hepatotoxic dose of CdCl₂(3.1 mg/kg, iv). Values represent the mean ± SE of 16-25 mice. Asterisk indicates significant difference from controls (p<0.05). (From Reference [15]).

Fig. 2. Sorbitol dehydrogenase (SDH) activities in control and MT-null mice 16 hr after injection of a hepatotoxic dose of CdCl₂(25 µmol/kg, ip). Values are mean ± SE(n=16-24). *Significantly different from control mice, p<0.05; "a" indicates a significant difference between CdCl₂ and Zn+CdCl₂ groups, p<0.05. (From Reference[17]).
have been produced by inserting a slightly modified mouse MT-I* gene into a pre-engineered MT 5'3' vector, which includes 10 and 7 kb of DNA flanking the MT-II and MT-I genes, respectively [10]. MT-I and II knock-out (MT-null) mice have also been produced by inserting sequences into MT-I and II-containing vectors to inactivate MT protein translation[11,12]. The modified genes contain sequences that either cause a frameshift mutation (MT-I) or early translation termination (MT-II). These genetically altered animals are visually indistinguishable from their corresponding controls. MT-transgenic mice have higher constitutive MT proteins in their tissues, whereas MT-null mice are deficient in MT, and cannot be induced in response to Cd exposure [13,14]. Hepatic cytochrome P-450 enzymes and antioxidant components in MT-transgenic animals appear to be the same as controls [13,14]. In this review, we discuss the use of these MT-transgenic and MT-null mice to study the role of MT in Cd toxicology.

**Use of MT transgenic and null mice to study the role of MT in Cd lethality and hepatotoxicity**

To determine the role of MT in Cd-induced lethality, MT-transgenic and null mice were given a wide range of Cd doses, and lethality was determined 24 hr later. MT-transgenic mice were more resistant to Cd-induced lethality than control mice. For example, Cd(3.7 mg/kg) was lethal to 73% of control mice, but only to 13% of MT-transgenic mice [15]. In comparison, MT-null mice were more sensitive to Cd-induced lethality than corresponding controls [11,12,16,17]. These data indicate that MT concentration is an important mechanism preventing the lethal effects of Cd.

The liver accumulates substantial amounts of Cd after acute Cd administration [14-19], and liver injury is prominent only 5 hrs after Cd administration [18,19], as evidenced histologically by congestion, apoptosis, necrosis and peliosis [15-19]. Cd-induced liver injury is so severe that hepatic failure is thought to be responsible for acute Cd lethality [15,18,19].

The induction of MT by either Cd or Zn has been shown to protect against Cd-induced liver injury. The mechanism of the protection appears to be due to intracellular sequestration of Cd to metallothionein(CdMT), thus reducing the interaction of Cd with target molecules [20]. To confirm this hypothesis, MT-transgenic mice were studied. Cd administration(3.1 mg/kg, iv) to control mice produced extensive liver injury, as evidenced by 20- and 70-fold increases in serum enzyme activities of sorbitol dehydrogenase and alanine aminotransferase, respectively. MT-transgenic mice were considerably more resistant to Cd-induced hepatotoxicity than control mice, as evidenced by the elevation in serum enzymes being only about one-tenth that observed in control mice, and a lower incidence of hepatic necrosis(Fig.1).

To ascertain the mechanism of the protection, distribution of Cd to various organs, and subcellular distribution of Cd in liver was determined. No difference in organ distribution of Cd was evident between control and MT-transgenic mice. However, hepatic subcellular distribution of Cd was altered markedly in MT-trans-
genic mice, with much less Cd distribution to nuclei, mitochondria, and microsomes (25, 42, and 24% of controls, respectively), and more Cd distributing to the cytosol (240% of controls). The increased cytosolic Cd was bound primarily to MT, as determined by G-75 gel chromatography.

The protective role of Zn-induced MT against Cd hepatotoxicity is further demonstrated using MT-I and -II null mice. Zn pretreatment of control mice increased hepatic MT concentration 80 fold, and protected against CdCl2-induced liver injury. Of course, Zn treatment cannot increase MT in MT-null mice, and failed to protect against Cd hepatotoxicity (Fig.2). These data indicate that Zn-induced protection against CdCl2-induced hepatotoxicity appears to be mediated, at least in part, by Zn induction of hepatic MT.

Use of MT transgenic and null mice to study the role of MT in Cd NEPHROTOXICITY

Although MT plays an important role in acute Cd hepatotoxicity, it is doubtful whether MT can provide long-term protection against Cd toxicity. With long-

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<tr>
<th>Effects</th>
<th>MT-transgenic</th>
<th>MT-null</th>
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<td>Cd-induced MT induction</td>
<td>↑↑↑</td>
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<tr>
<td>Cd absorption from the GI tract</td>
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<td>Cd distribution to tissues</td>
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<td>Cd elimination from the body</td>
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<td>Cd-induced acute hepatotoxicity</td>
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<td>Cd-induced c-Jun and p53 mRNA expression</td>
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<td>CdMT-induced acute nephrotoxicity</td>
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<td>Cd-induced chronic nephrotoxicity</td>
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<td>Susceptibility to oxidative stress</td>
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Compared to corresponding controls: ↑ indicates increase; ↔ indicate no effects; ↓ indicates decrease; N.D. not determined.
term low-level exposure to Cd, hepatotoxicity is rarely reported and nephrotoxicity is the major concern[21].

Following chronic CdCl2 exposure (up to 10 weeks), MT-null mice are more susceptible than controls to Cd-induced nephrotoxicity, as evidenced by blood urea nitrogen and proteinuria (Fig.3), as well as by glucosuria, enzymuria and histopathology. Renal MT concentrations increased 100-fold in control mice, but are unchanged in MT-null mice, suggesting that induction of renal MT is also an important adaptive mechanism in decreasing chronic Cd-induced renal injury.

The CdMT complex is known to be nephrotoxic when injected intravenously to laboratory animals. As a result, it has been suspected that release of CdMT from the liver and uptake by the kidney is responsible for the chronic toxicity of Cd[21-239]. However, using these MT-null mice, we demonstrate that Cd-induced nephrotoxicity is not necessarily mediated through CdMT complex. Furthermore, the discrepancies in regards to the role of MT in Cd nephropathy between a single CdMT injection[17,24] and chronic CdCl2 exposure (Fig.3), leads us to hypothesize that a single injection of CdMT is not a good model to study chronic Cd nephropathy. This hypothesis is further supported by extensive histopathology examinations (Data not shown).

The role of MT in Cd toxicity has become clearer by the use of MT-I transgenic and MT-I/II knockout mouse models. Due to the page limitations, our major findings are summarized in Table 1 below.

The MT-transgenic and knockout mouse models provide complementary approaches to the questions of interest, and have greatly increased our understanding of the role of MT in Cd toxicity, as well as other biological functions of MT.

ACKNOWLEDGMENTS

This work was supported by NIH grant ES-01142 and ES-07079.

We thank Drs. S. Habeebu, M.B. Iszard, Y.P. Liu, and H. Zheng for their contribution to the projects. We also thank Drs. G.K. Andrews, K.H.A. Choo, and R.D. Palmer for their collaboration in these studies.

REFERENCES


