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Use of genetically altered animal models in understanding the role of metallothionein in cadmium toxicity*

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Abstract: Acute Cd exposure produces liver injury, whereas chronic Cd exposure damages the kidney but not the liver. Previous experiments suggest that the low-molecular-weight, metal-binding protein metallothionein (MT) in liver protects against liver injury, but is responsible for the kidney injury observed after chronic Cd exposure. Thus, prior to the development of MT-transgenic and MT-knockout mice models, MT's role was always assumed to be a toxicological paradox, hepatoprotection but nephrotoxicity. The development of MT-transgenic and MT-knockout mice models has reconfirmed MT's protective role against Cd-induced hepatotoxicity, but it has challenged MT's suggested role in Cd-induced nephrotoxicity. In this communication, recent data using these genetically altered mice models indicate that MT protects against not only the Cd-induced hepatotoxicity, but also nephrotoxicity, hematotoxicity, immunotoxicity, and bone damage.

INTRODUCTION

Metallothioneins (MTs) are low-molecular-weight, cysteine-rich, metal-binding proteins that are inducible to varying degrees (moderate to very high) by a plethora of physicochemical agents. MTs have been found throughout the animal kingdom, in higher plants, in eukaryotic microorganisms, and also in many prokaryotes. Based on their structural similarities, MTs have been divided into three classes: class I, class II, and class III. Class I MTs, which include mammalian MTs, are the focus of this review.

The discovery of MT by Margoshes and Vallee [1] signaled the beginning of a long trail of research that, during the last 40 years, has generated a wealth of information about its structure and regulation. Its real biological function, however, has remained almost as enigmatic as when it was first discovered. The spectrum of suggested biological roles for MT was so wide that up until the development of MT-transgenic (MT-TG) and MT-knockout (MT-null) mice, it seemed to be an essential protein for life. A diverse range of biological functions have been suggested, ranging from metal homeostasis and heavy metal detoxification, protection against oxidative stress, control of gene expression, including developmental regulation, neuroprotection, carcinogenesis, and acting as a chaperone for metalloproteins. [2,3].

The development of MT-TG and MT-null mice has provided unique models, and studies using these models have provided new clues that should help reshape the current thinking on MT's function. In this communication, we discuss some of the work that have been performed using MT-TG and MT-null mice models, and the impact of these finding in reconfirming as well as challenging some of the current dogma on MT's biological functions.

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Role of metallothionein in protection against cadmium-induced hepatotoxicity

The correlation between cellular MT levels and Cd resistance has been extensively documented. Pretreatment of animals with any chemical (including low doses of Cd) that induces hepatic MT levels, protects the animal from acute Cd-induced hepatotoxicity and lethality. The mechanism appears to be due to sequestration of Cd in the cytosol by MT, thereby preventing its distribution in critical subcellular compartments [4]. MT's protective role against Cd-induced hepatotoxicity was further supported by studies in newborn rats. Newborn rats have very high constitutive levels of hepatic MT, and are resistant to Cd-induced hepatotoxicity [5,6]. Cultured cells expressing high levels of MT are also resistant to Cd-induced hepatotoxicity [7].

Use of MT-TG and MT-null mice in recent years reconfirmed MT's role in protection against Cd-induced hepatotoxicity and lethality. MT-TG transgenic mice, which have concentrations of hepatic MT ten-fold higher than that of control mice, are resistant to Cd-induced hepatotoxicity and lethality [8]. At a dose of 3.7 mg Cd/kg, only 27% of the control animals survived while 87% of the transgenic animals survived. Consistent with the high MT concentrations, the subcellular distribution of Cd also showed a greater (more than two-fold) accumulation in cytosol of MT-TG mice compared to control mice. Surprisingly, higher concentrations of MT in MT-TG mice do not appear to inhibit the gastrointestinal absorption of Cd when administered orally, nor does it alter the organ distribution of Cd [9].

Consistent with the results in MT-TG mice that are resistant to Cd toxicity due to higher MT expression levels, MT-null mice were found sensitive to Cd-induced lethality and hepatotoxicity [10]. Hepatocytes from MT-null mice were also sensitive to Cd-induced cytotoxicity [11]. Lack of MT in MT-null mice also translated into secondary effects on Cd-induced gene expression in the liver. Zheng *et al*. [12] showed that the magnitude of induction of c-jun and p53 mRNA was more pronounced in MT-null mice compared to controls, and the elevated mRNA levels were seen at lower doses of Cd in MT-null mice (10 mmol/kg in MT-null vs 40 mmol/kg in controls). This indicates that MT may indirectly modulate other biological effects of Cd by restricting its availability.

The studies with MT-TG and MT-null mice therefore reinforced a protective role of MT against Cd-induced hepatotoxicity.

Role of metallothionein in protection against Cd-induced bone damage, hematotoxicity, and immunotoxicity

It was recently reported from our laboratory [13] that chronic cadmium administration leads to a dose-dependent decrease, up to 25%, in bone mass. Loss of bone mass was due to a progressive decrease in bone density (osteoporosis). All these effects were significantly enhanced in MT-null mice. Furthermore, loss of bone mass in MT-null mice was detected at doses that were too low to produce detectable bone-mass loss in wild-type mice. This strongly suggests that lack of MT makes animals more vulnerable to Cd-induced osteoporosis and osteotoxicity. Thus, MT may play a protective role in Cd-induced bone damage.

Studies from our laboratory using MT-null mice also indicate that MT may play a protective role in hematotoxicity and immunotoxicity. Anemia is a common finding in animals after both oral and parenteral exposure to Cd [14]. Subcutaneous administration of Cd daily produced anemia in 5 weeks, as evidenced by decreased erythrocyte count, hemoglobin, mean corpuscular volume, etc., and all these effects were accentuated in MT-null mice compared to control mice [15]. Cd was also toxic to the immune system; it caused splenomegaly and thymic atrophy. It also increased serum level of IL-1β and TNF-α. The increased levels of these proinflammatory cytokines are probably responsible for Cd-induced inflammation and toxicity. All these hematotoxic and immunotoxic effects were increased in MT-null mice [15]. MT-null mice showed marked elevation of serum IL-1β and TNF-α levels at Cd doses that had no effect in control mice, again indicating the role of MT in attenuating these effects.

The results therefore strongly indicate that MT plays a protective role against Cd-induced bone damage and hematotoxic effects.

Role of metallothionein in the modulation of Cd-induced nephrotoxicity: A paradigm reversal

The most surprising and unexpected results recently obtained in our laboratory using MT-null mice, call for a reevaluation of the current theory on the role of MT in Cd-induced renal toxicity. The current model depicts that in the event of chronic Cd exposure, it is not the inorganic Cd in the plasma that is taken up by the kidney, but the Cd-MT complex that forms in the liver. This Cd-MT leaks out of the liver into the plasma, because of the damage to the hepatocytes. The circulating Cd-MT complex is taken up by the kidney where the MT is degraded in lysosomes and Cd is released. This Cd then binds to preformed MT in the kidney. When a critical concentration of Cd is reached in the kidney (~200 mg Cd/g wet weight), the MT becomes saturated with Cd, and renal injury occurs. Hence the protective role of MT in long-term Cd toxicity appeared to be contradictory, saving the liver but damaging the kidney [2]. To critically evaluate the role of MT in long-term toxicity, MT-null mice were exposed chronically to Cd for 10 weeks, after which kidney function and kidney morphology were examined [16]. Contrary to expectations, MT-null mice were found to have increased renal injury. In fact, the kidney of MT-null mice had a lower concentration of Cd compared to wild-type mice, but the extent of renal injury in MT-null mice was much greater. If the Cd-MT complex were responsible for the renal injury as depicted by the prevailing dogma, MT-null mice should have been less sensitive to renal injury because of the lack of functional MT and consequent failure to form the Cd-MT complex in liver. The increased susceptibility of MT-null mice is most likely due to their inability to synthesize MT in response to Cd exposure, suggesting strongly a protective role of metallothionein against Cd-induced nephrotoxicity.

Thus, use of MT-TG and MT-null mice has helped reverse the current dogma on the role of MT in Cd-induced nephrotoxicity.

CONCLUSION

MT-transgenic and MT-null mice are unique models that not only have helped change existing paradigm on the role of MT in nephrotoxicity, but also have raised new questions on the importance of MT in life and its evolution. Studies using MT-TG and MT-null mice strongly indicate that the suggested protective functions of metallothionein other than protection against Cd are less than convincing [17–22]. It appears that MT became more important and indispensable for protection against Cd and possibly other heavy metals than performing other suggested protective functions. Because the half-life of Cd is 10–40 years in the body while that of other metals is in months, MT's role in protection against Cd toxicity appears to be much more important and significant throughout the life span of an individual.

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