Protective role of metallothionein (I/II) against pathological damage and apoptosis induced by dimethylarsinic acid

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Abstract

AIM: To better clarify the main target organs of dimethylarsinic acid toxicity and the role of metallothionein (MTs) in modifying dimethylarsinic acid (DMAA) toxicity.

METHODS: MT-I/-II null (MT-/-) mice and the corresponding wild-type mice (MT+/-) six in each group were exposed to DMAA (0-750 mg/kg body weight) by a single oral injection. Twenty four hours later, the lungs, livers and kidneys were collected and undergone pathological analysis, induction of apoptotic cells as determined by TUNEL and MT concentration was detected by radio-immunoassay.

RESULTS: Remarkable pathological lesions were observed at the doses ranging from 350 to 750 mg/kg body weight in the lungs, livers and kidneys and MT-/- mice exhibited a relatively slight destruction when compared with that in dose matched MT+/- mice. The number of apoptotic cells was increased in a dose dependent manner in the lungs and livers in both types of mice. DMAA produced more necrotic cells rather than apoptotic cells at the highest dose of 750 mg/kg, however, no significant increase was observed in the kidney. Hepatic MT level in MT-/- mice was significantly increased by DMAA in a dose-dependent manner and there was no detectable amount of hepatic MT in untreated MT-/- mice.

CONCLUSION: DMAA treatment can lead to the induction of apoptosis and pathological damage in both types of mice. MT exhibits a protective effect against DMAA toxicity.

Jia G, Gu YQ, Chen KT, Lu YY, Yan L, Wang JL, Su YP, Wu JCG. Protective role of metallothionein (I/II) against pathological damage and apoptosis induced by dimethylarsinic acid. World J Gastroenterol. 2003; 10(1): 91-95
http://www.wjgnet.com/1007-9327/10/91.asp

INTRODUCTION

Arsenic is a metalloid that naturally occurs in soil, water, and air. Arsenicals are also non-biodegradable by-products during production of copper, lead, and other ores and coal consumption. Exposure to arsenic by food, drinking water, soil and air containing arsenic is widely existed in the world. Inorganic arsenicals are well known human carcinogens, specifically for the lung, liver, kidney, skin, bladder and other internal organs[2,24]. Dimethylarsinic acid (DMAA) is a major form of organic arsenic in the environment and the main metabolite of ingested inorganic arsenicals in most mammals, including humans[2,24]. DMAA itself can be used as herbicide and pesticide and also naturally exists in some seafood. Recent studies have revealed that DMAA is a genotoxic, multi-site promoter of carcinogenesis as well as a complete carcinogen in rodents[21,25], which provides a novel clue to investigate the mechanism of arsenicals in carcinogenesis.

Arsenicals, including DMAA, are moderately effective inducers of MT in mice and rats[16-20]. MTs, thiol-rich metal binding proteins, have been shown to be easily induced by oxidative stress and heavy metals and play an important role in homeostasis of essential metals, detoxication of heavy metals, scavenging reactive oxygen intermediates and preventing oxidative stress and heavy metals and especially to be mentioned, its capacity of scavenging hydroxyl and superoxide radicals is much more efficient than GSH, an established antioxidant[15]. Among the four major isoforms of identified MTs, MT-I and MT-II existing in all tissues examined, are the predominant forms in the livers. Recently Liu et al[26] reported that MT-I/II null mice were more sensitive than wild type mice to hepatotoxic and nephrotoxic effects of oral or injected inorganic arsenicals. Sakurai et al[27] reported that DMAA could induce apoptosis by reducing glutathione (GSH) in vitro. However, the effect of MT on induction of apoptosis and the main organic toxicity by DMAA in vivo remain elusive.

MT-I/II null (MT-/-) mice have been proved to be a good tool for studying MT’s normal function and the consequences of its deficiency[28]. In the present study, MT-I/II null (MT-/-) mice and the corresponding wild-type mice (MT+/-) were exposed to DMAA by oral injection, we investigated the pathological lesions and apoptosis in main target organs including the liver, lung and kidney of the mice, to elucidate the toxicity of DMAA and the ability of MT to modify DMAA toxicity.

MATERIALS AND METHODS

Chemicals

Dimethylarsinic acid (purity 100 %) was purchased from Wako Pure Chemical Co. (Osaka, Japan). An in situ apoptosis detection kit (ApopTag™) was purchased from Intergen Co. NY, USA.

Animals and treatment

MT null (MT-/-) mice whose MT-I and II genes had null mutation and wild type (MT+/-) mice provided kindly by Dr. A. Choo (Murdoch Institute for Research into Birth Defects, Royal Children’s Hospital, Australia), were of a mixed genetic background of 129 Ola and C57BL/6 strains. F1
hybrid mice were mated with C57BL/6 mice, and their
offsprings were back-crossed to C57BL/6 for six generations.
MT−/− and MT+/+ mice were obtained by mating of those heterozygous (MT+/−) mice.

MT−/− and MT+/+ mice were routinely bred in the vivarium of the National Institute for Environmental Studies (NIES, Japan). Microbiological and viral examinations were performed with regular quarantine procedures for more than one year, and we did not find either pathogenic infections or significant phenotypical abnormalities. Both strains of mice were housed in cages in ventilated animal rooms with a controlled temperature of 23±1 °C, a relative humidity of 55±10%, and a 12 h light/dark cycle. They were maintained on standard laboratory chow and tap water ad lib, and received humane care throughout the experiment according to the guidelines of the NIES. Eight-week-old female MT−/− and MT+/+ mice were assigned randomly in equal numbers to all groups (six mice for each treatment group). Fresh DMAA solution was prepared by dissolving it in sterilized water. The mice were administered DMAA (0-750 mg/kg) by oral gavage.

**Sample collection**

At 24 h after administration of DMAA, the lung, liver and kidney were collected from each mouse under diethyl ether anesthesia. Portions of tissues were fixed in 10% neutral formalin, processed by the standard histological techniques, and stained with hematoxylin and eosin for light microscopic examination. For TUNEL staining, sections (5 µm) were placed on poly-L-lysine precoated slides.

**TUNEL for apoptosis**

Apoptotic cells were detected with an apoptosis detection kit according to the manufacturer’s instructions. Briefly, the samples were incubated with digoxigenin-labeled dNTP in the presence of terminal deoxynucleotidyl transferase followed by peroxidase-conjugated anti-digoxigenin antibody. Nuclear staining of apoptotic cells was detected with 3′, 3′-diaminobenzidine followed by counterstaining of nuclei with methyl green. An apoptosis index (AI) was obtained by dividing the number of positive cells in the area observed.[19]

**MT Concentration**

MT (MT-I and MT-II isoforms) concentration in the liver was measured by radioimmunoassay using sheep anti-rat MT-I antiserum.[20] The detection limit of this method was 0.2 µg MT/g of tissue.

**Statistical analysis**

ANOVA with subsequent post hoc’s test was used as appropriate. All values were expressed as ±S. Differences were considered significant at P<0.05.

**RESULTS**

**Histopathological observation**

In untreated MT−/− mice and the corresponding MT+/+ mice, the lung, liver and kidney showed normal morphology. Significant lesions were observed at doses of DMAA ranging from 375 to 750 mg/kg body weight in both types of mice. However, the pathological lesions in MT−/− mice were more severely widespread when compared to that in dose matched MT+/+ mice.

Changes including congestion, atelectasis and mild to moderate hemorrhages in the alveoli of the lungs were observed in MT−/− mice. Adequate air space in the alveoli was observed more frequent in MT+/+ mice compared to that of MT−/− mice. Pulmonary capillary congestion could affect alveolar space, resulting in severe acute impairment of respiratory function.

Capillary rupture led to leakage of red blood cells into the interstitium, as well as into the alveoli (Figure 1).

**Figure 1** Typical HE staining. The bar is 100 µm. A, B: the lungs from control of MT−/− and MT+/+ mice; C, D: the lungs from 750 mg/kg DMAA group of MT−/− and MT+/+ mice. Arrows indicate atelectasis and hemorrhage.

At 24 h after DMAA treatment, severe liver damages characterized by cellular cloudy swelling, paleness of cell cytoplasm, vacuolization of hepatocytes and a few areas of focal necrosis were found in MT−/− mice while a limited degree of changes was observed in dose matched MT+/+ mice livers (Figure 2).

**Figure 2** Typical HE staining. The bar is 100 µm. A, B: the livers from control of MT−/− and MT+/+ mice; C, D: the livers from 750 mg/kg DMAA group of MT−/− and MT+/+ mice. The arrows indicate necrosis.
Histological changes in the kidney are shown in Figure 3. Treatment with DMAA produced swelling of glomerulus and its surrounding tubular tissue and urinary space compression in both types of mice.

**Figure 3** Typical HE staining. The bar is 100 µm. A, B: the kidneys from control of MT⁻/⁻ and MT⁺/+ mice; C, D: the kidneys from 750 mg/kg DMAA group of MT⁻/⁻ and MT⁺/+ mice. The arrows indicate the swelling of glomerulus and the surrounding tubular tissue and urinary space compression.

188 mg/kg body weight. Brown staining indicates apoptotic cells. The bar is 20 µm. A1 in the livers. All values were expressed as t±s. ANOVA with subsequent post hoc’s test was performed for comparison of A1. a,b Significant difference at P <0.05, P <0.01 when compared with the corresponding control group. c,d Significant difference at P <0.05, P <0.01 when compared with the dose-matched MT⁻/⁻ mice group.

**Figure 4** Apoptosis in lungs of MT⁺/+ and MT⁻/⁻ mice detected by TUNEL twenty-four hours after oral DMAA treatment. A: Typical apoptotic cells in alveolar area of MT⁻/⁻ mice at a dose of 188 mg/kg body weight. Brown staining indicates the apoptotic cells. The bar is 20 µm. B: Typical apoptotic cells in bronchial area of MT⁻/⁻ mice at a dose of 188 mg/kg body weight. Brown staining indicates apoptotic cells. The bar is 20 µm. C: AI in alveolar area. D: AI in bronchial area. All the values were expressed as t±s. ANOVA with subsequent post hoc’s test was performed for comparison of AI. a,b Significant difference at P <0.05, P <0.01 when compared with the corresponding control group. c,d Significant difference at P <0.05, P <0.01 when compared with the dose-matched MT⁻/⁻ mice group.

**Figure 5** Apoptosis in livers of MT⁺/+ and MT⁻/⁻ mice detected by TUNEL twenty-four hours after oral DMAA treatment. A: Typical apoptotic cells in the liver of MT⁻/⁻ mouse at a dose of 188 mg/kg body weight. Brown staining indicates apoptotic cells. The bar is 20 µm.
Induction of apoptotic cells detected in lungs, livers and kidneys of MT<sup>+</sup> and MT<sup>−/−</sup> mice

High induction of apoptotic cells in bronchial epithelial cells was observed in MT<sup>−/−</sup> mice treated with DMAA at 375 mg/kg body weight; however, the same changes were not observed in dose matched MT<sup>+/+</sup> mice. At a high dose of 750 mg/kg body weight, the coincident increase of apoptotic cells was observed in both types of mice and no significant difference was observed between them.

In control group, the incidence of apoptotic cells in alveolar epithelial cells in MT<sup>−/−</sup> mice was significantly higher than that in MT<sup>+/+</sup> mice, implying that MT<sup>−/−</sup> mice might have a stronger ability to induce apoptosis than MT<sup>+/+</sup> mice. A significant increase of apoptotic cells occurred in MT<sup>−/−</sup> mice treated by 188 mg/kg DMAA, a relative low dose when compared with that in bronchial epithelial cells. However, no significant increase was observed in MT<sup>−/−</sup> mice at the same dose of 188 mg/kg DMAA. With the increase of dose, high induction of apoptotic cells was observed in both types of mice (Figure 4).

Figure 5 shows that in control group, the incidence of apoptotic cells in MT<sup>−/−</sup> mice was 156.3±41.0/cm², significantly higher than that in MT<sup>+/+</sup> mice. The incidence of apoptotic cells in the livers rose with the dose of dose in both types of mice. However, at the highest dose of 750 mg/kg, DMAA produced more necrotic cells rather than apoptotic cells observed by HE staining (Figure 2).

MT concentration in liver of MT<sup>−/−</sup> mice

MT concentration was determined in the liver of MT<sup>+/+</sup> mice and MT<sup>−/−</sup> mice treated with DMAA (Figure 6). Hepatic MT level in MT<sup>−/−</sup> mice was significantly increased by DMAA in a dose-dependent manner. However, there was no detectable amount of hepatic MT in untreated MT<sup>−/−</sup> mice, and it could not be induced by DMAA.

**Figure 6** MT concentration in livers of MT<sup>+/+</sup> mice detected by radio-immunoassay. All the values were expressed as x±s.

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**ACKNOWLEDGEMENT**

We thank Dr. Sone Hideko and Masahiko Satoh (National Institute for Environmental Studies, Japan) for kindly providing the experiment materials. This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, China and a grant from the Japan Science and Technology Agency, Japan.
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Edited by Zhu LH and Wang XL