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World J Gastroenterol 2004;10(1):91-95
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

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

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Received: 2003-07-04 **Accepted:** 2003-07-24

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[1,2]. 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[2-4]. 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[5-7], 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[8,9]. 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 carcinogenesis as an endogenous defensive factor[10-15]. 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*[16] 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*[17] 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[18]. 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 ℃, 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 $\bar{x}\pm s$. 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).

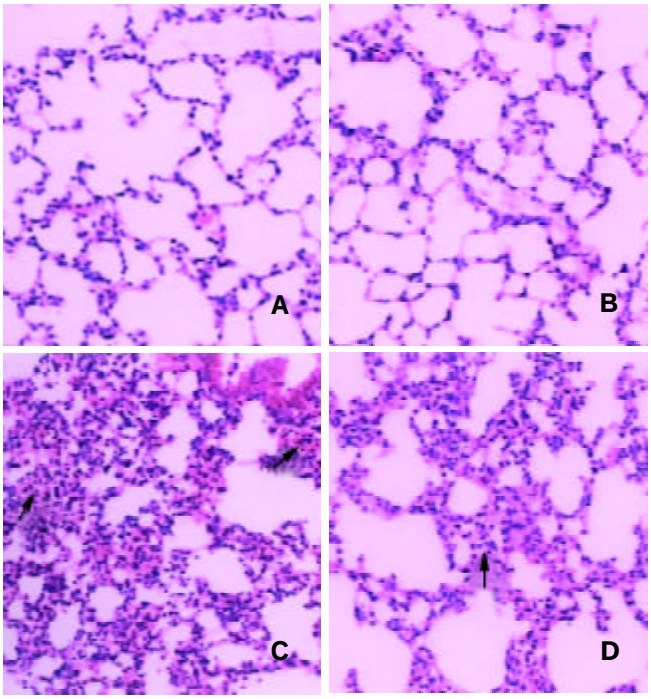

Figure 1 Typical HE staining. The bar is 100 μm. A, B: the lungs from control of $MT^{-/-}$ and $MT^{+/+}$ mice; C, D: the lungs from 750 mg/kg DMAA group of $MT^{-/-}$ and $MT^{+/+}$ mice. Arrows indicate atelectasis and hemorrhage.

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).

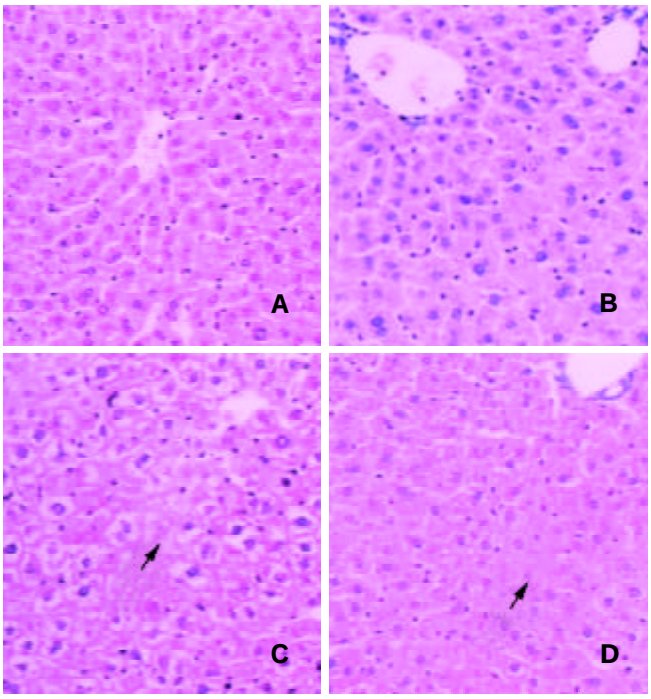

Figure 2 Typical HE staining. The bar is 100 μm. A, B: the livers from control of $MT^{-/-}$ and $MT^{+/+}$ mice; C, D: the livers from 750 mg/kg DMAA group of $MT^{-/-}$ and $MT^{+/+}$ mice. The arrows indicate necrosis.

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.

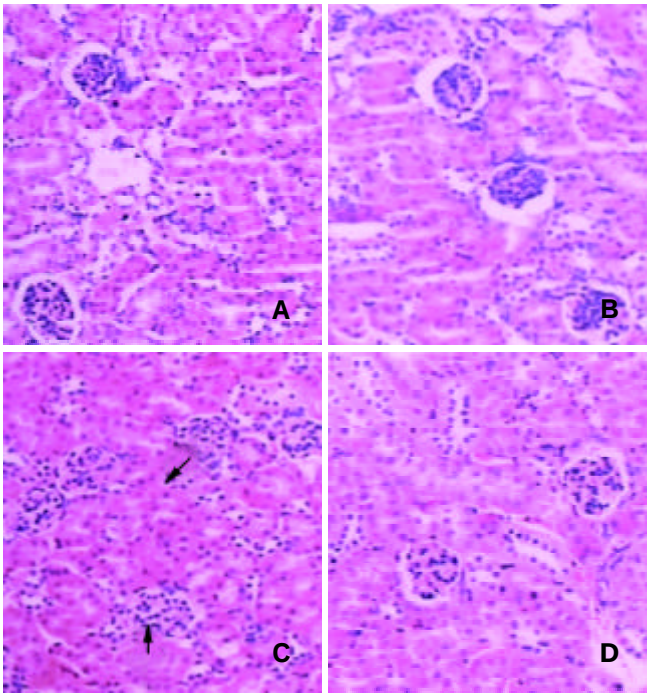

Figure 3 Typical HE staining. The bar is 100 μm. A, B: the kidneys from control of $MT^{-/-}$and $MT^{+/+}$ mice; C, D: the kidneys from 750 mg/kg DMAA group of $MT^{-/-}$and $MT^{+/+}$ mice. The arrows indicate the swelling of glomerulus and the surrounding tubular tissue and urinary space compression.

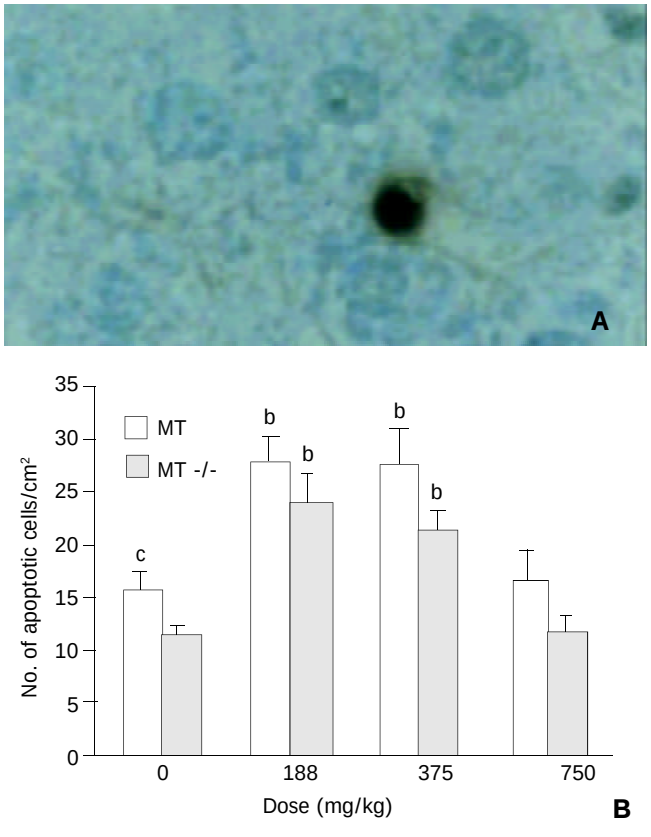

Figure 5 Apoptosis in livers of $MT^{+/+}$ and $MT^{-/-}$ mice detected by TUNEL twenty-four hours after oral DMAA treatment. A: Typical apoptotic cells in the liver of $MT^{-/-}$ mice at a dose of 188 mg/kg body weight. Brown staining indicates apoptotic cells. The bar is 20 μm. B. AI in the livers. All values were expressed as $\bar{x}\pm s$. 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.

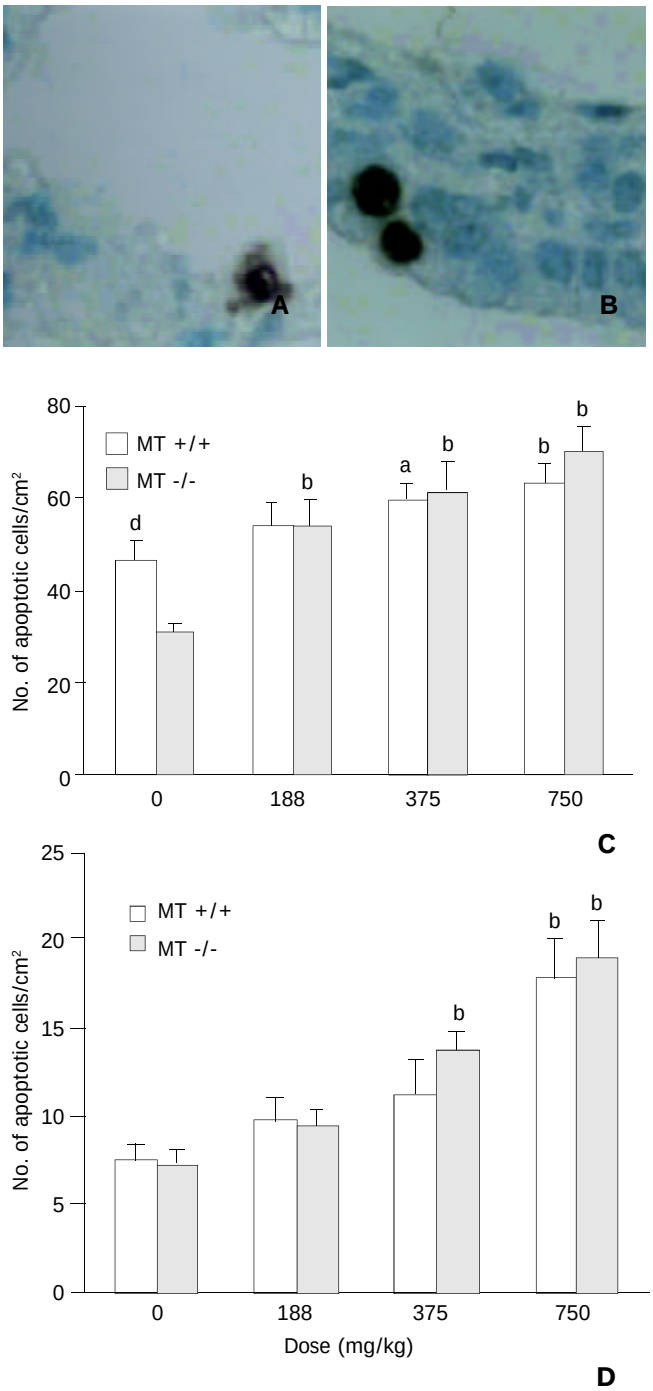

Figure 4 Apoptosis in lungs of $MT^{+/+}$ and $MT^{-/-}$ mice detected by TUNEL twenty-four hours after oral DMAA treatment. A: Typical apoptotic cells in alveolar area of $MT^{-/-}$ mice at a dose of 188 mg/kg body weight. Brown staining indicates the apoptotic cells. The bar is 20 μm. B: Typical apoptotic 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 $\bar{x}\pm s$. 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.

Induction of apoptotic cells detected in lungs, livers and kidneys of MT$^{-/-}$ and MT$^{+/+}$ mice

High induction of apoptotic cells in bronchial epithelial cells was observed in MT$^{-/-}$ mice treated with DMAA at 375 mg/kg body weight, however, the same changes were not observed in dose matched MT$^{+/+}$ 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$^{+/+}$ mice was significantly higher than that in MT$^{-/-}$ mice, implying that MT$^{+/+}$ mice might have a stronger ability to induce apoptosis than MT$^{-/-}$ mice. A significant increase of apoptotic cells occurred in MT$^{-/-}$ 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$^{+/+}$ 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$^{+/+}$ mice was 156.33±41.041/cm^2, significantly higher than that in MT$^{-/-}$ mice. The incidence of apoptotic cells in the livers rose with the increase 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).

DMAA failed to induce remarkable apoptotic cells in the kidneys from both types of mice (data not shown).

MT concentration in liver of MT$^{+/+}$ mice

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

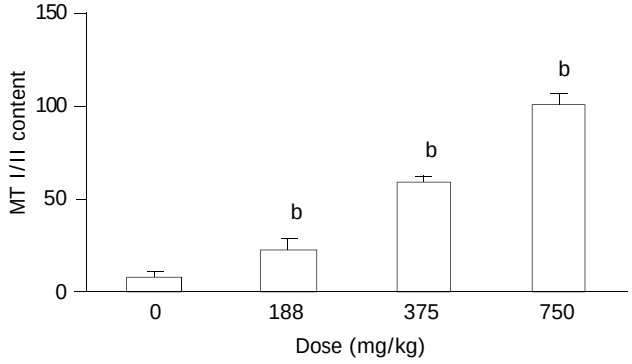

Figure 6 MT concentration in livers of MT$^{+/+}$ mice detected by radio-immunoassay. All the values were expressed as $\bar{x}\pm s$. ANOVA with subsequent *post hoc's* test was performed for comparison of AI. ^{b}Significant difference at $P<0.01$ when compared with the corresponding control group.

DISCUSSION

The present study demonstrated that DMAA could produce pathological lesions in the lungs, livers and kidneys, and induce apoptosis in the lungs and livers. Most importantly, it is the first report to show that inability to produce MT-I/II in MT$^{-/-}$ mice caused an increased sensitivity to toxicity induced by DMAA.

Treatment with DMAA caused severe and wide spread lesions in MT$^{-/-}$ mice, whereas, these changes were much less severe in MT$^{+/+}$ mice. It indicates that MT$^{-/-}$ mice were more sensitive to DMAA and MT played a protective role against the toxicities to main organs. Yamanaka[7] reported that DMAA in mice could be further metabolized and converted into dimethylarsine radicals and dimethyl arsenic peroxy radicals. Marked formation of 8-oxod G was observed in the lung and liver, which are the target organs for arsenic carcinogenesis. No increase in 8-oxod G levels was observed in the kidney. Meanwhile, MT was capable of scavenging hydroxyl and superoxide radicals and its capability of scavenging them was much more efficient[11,12,15]. In our study, the expression of MT (I/II) was induced by DMAA in a dose dependent manner in the livers of MT$^{+/+}$ mice, no MT(I/II) was observed in MT$^{-/-}$ mice. Thus the reduction of lesions induced by DMAA in the main target organs of MT$^{+/+}$ mice could be explained at least partly by MT reduction induced by DMAA, and the toxicity induced by DMAA could be explained partly by way of oxidative stress participation.

After a lethal damaging stimulation, two main structural routes of cell death might occur: apoptosis and necrosis. It has become apparent that the magnitude and type of injurious stimuli could determine whether a cell underwent death through apoptosis or necrosis. Severe damaging stimuli tended to result in necrosis, and lower grade damaged stimuli tended to cause apoptosis[21-30].

Recently, Sakurai *et al* reported that DMAA could induce apoptosis by reducing glutathione (GSH) *in vitro*[17]. However, the effect of MT on the induction of apoptosis by DMAA *in vivo* remains elusive. Furthermore, the perturbation of apoptosis has been thought to contribute to carcinogenesis either through enhanced initiation or progression[29-38]. In this study, the induction of apoptosis was detected by TUNEL in the lungs, livers and kidneys from both types of mice. In the lungs, a significant increase of apoptosis was observed in alveolar cells of MT$^{-/-}$ mice at a relative low level compared with that in bronchial cells, suggesting that alveolar cells were more sensitive than bronchial cells and MT had some protective role against the induction of apoptosis induced by DMAA at a relative low level. With the increase of dose, DMAA induced high levels of apoptosis in both types of mice and at the highest dose of 750 mg/kg, necrotic cells predominated over apoptosis in the livers, revealing the serious toxicity of DMAA at this dose. Since the kidney is the major organ for arsenic elimination and most of arsenicals could be rapidly eliminated through the kidney[1-3], renal cells are thus exposed to a major portion of the absorbed arsenical dose. However, the induction of apoptosis was not affected by DMAA, the underlying mechanism needs further investigations.

In conclusion, the present studies demonstrate that oral administration of DMAA can produce toxic response of the respiratory system, liver and kidney in both MT$^{-/-}$ and MT$^{+/+}$ mice. The pathological effects are clearly pronounced in MT$^{-/-}$ mice. Intracellular MT appears to play an important role in preventing the toxic effects of DMAA.

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