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Renal Toxicity Caused by Cisplatinum in Glutathione-Depleted Metallothionein-Null Mice

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ABSTRACT. To elucidate the protective role of metallothionein (MT) and glutathione (GSH) in renal toxicity caused by cisplatinum (*cis*-DDP), we examined the sensitivity of GSH-depleted MT-null mice to the renal toxicity of *cis*-DDP. Blood urea nitrogen and creatinine values in the serum, and histopathological change in the kidney were utilized as indicators of nephrotoxicity caused by *cis*-DDP. Although *cis*-DDP exerted renal toxicity in MT-null mice and wild-type mice, the toxicity was more conspicuous in the MT-null mice than in the wild-type mice. Moreover, renal toxicity caused by *cis*-DDP was enhanced significantly by a decrease in the renal GSH level by buthionine sulfoximine (BSO) pretreatment in both kinds of mice. The *cis*-DDP-caused nephrotoxicity that was enhanced by BSO-mediated GSH depletion was much more severe in the MT-null mice than in the wild-type mice. However, preadministration of zinc sulfate cancelled the BSO-enhanced, *cis*-DDP-dependent renal toxicity in the wild-type mice, but not in the MT-null mice. In the present study, we found that MT and GSH play an important, cooperative role in detoxification of severe kidney damage caused by *cis*-DDP. Moreover, the renal MT preinduced by zinc could protect mice from *cis*-DDP nephrotoxicity enhanced by GSH depletion. BIOCHEM PHARMACOL **60**;11:1729–1734, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. metallothionein; glutathione; cisplatinum; renal toxicity; knockout mice; buthionine sulfoximine

cis-DDP§ is one of the most extensively evaluated antineoplastic agents. It has potent antitumor activity with a broad anticancer spectrum against certain human neoplasms [1, 2]. However, *cis*-DDP produces severe side-effects in the kidney, bone marrow, and gastrointestinal system [3]. In particular, the clinical use of *cis*-DDP is hampered by its severe nephrotoxicity. Although the mechanism of renal toxicity caused by *cis*-DDP is still not clear, there is evidence that *cis*-DDP produces inter- and intra-strand cross-links in nucleic acid [4, 5], inhibits a number of sulfhydryl-containing enzymes [6, 7], and induces lipid peroxidation by the generation of free radicals [8, 9].

GSH, a cysteine-containing tripeptide, is considered as a protective cellular factor against *cis*-DDP toxicity. Administration of either GSH or GSH ester to laboratory animals has protected them from adverse effects caused by *cis*-DDP [10–13]. It has been established that the renal toxicity of *cis*-DDP is enhanced by depletion of tissue GSH by using BSO, a specific inhibitor of γ-glutamylcysteine synthetase [14, 15].

On the other hand, preadministration of MT-inducing metal such as bismuth can suppress the renal toxicity of *cis*-DDP [16–20]. MT is a cysteine-rich low-molecular-weight protein with a high affinity for metals such as cadmium, mercury, and platinum, and is induced by various metals and many other factors such as glucocorticoids and cytokines [21]. Recent studies have shown that the sensitivity to the renal toxicity of *cis*-DDP is increased in MT-null transgenic mice having a null mutation of *MT-I* and *-II* genes [22, 23].

Nakagawa and coworkers [24] found that pretreatment with a zinc compound attenuates BSO-enhanced, *cis*-DDP-dependent nephrotoxicity, and they speculated that this protective effect is due to preinduced MT. However, this hypothesis has not been validated because zinc shows not only MT induction but also other effects [25–27]. Moreover, the relationship between endogenous MT and GSH in the detoxification of *cis*-DDP has not been studied.

In the present study, we examined the sensitivity of GSH-depleted MT-null mice to renal toxicity of *cis*-DDP in order to elucidate the role of MT and GSH in *cis*-DDP-caused nephrotoxicity. We also explored the effect of pretreatment with zinc on *cis*-DDP nephrotoxicity caused by GSH depletion in the MT-null mice and the wild-type mice.

MATERIALS AND METHODS

Animals and Chemicals

MT-null ($MT^{-/-}$) mice whose *MT-I* and *-II* genes had null mutation and wild-type ($MT^{+/+}$) mice, which were pro-

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§ *Abbreviations*: *cis*-DDP, cisplatinum; GSH, glutathione; MT, metallothionein; BSO, buthionine sulfoximine; and BUN, blood urea nitrogen.

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TABLE 1. MT and GSH concentrations in the kidneys of MT-null mice and wild-type mice treated with BSO and zinc sulfate

Treatments	MT (μg/g tissue)		GSH (μmol/g tissue)	
	Wild-type	MT-null	Wild-type	MT-null
Untreated	2.94 ± 0.79	<0.2	4.00 ± 0.33	3.70 ± 0.54
BSO	7.43 ± 1.47*	<0.2	0.82 ± 0.30	0.84 ± 0.24
Zn + BSO	89.1 ± 14.4†	<0.2	0.81 ± 0.21	0.75 ± 0.19

MT-null mice and wild-type mice were given an injection (s.c.) of zinc sulfate (200 μmol/kg) once a day for 2 days. MT and GSH concentrations in the kidneys of these mice were determined at 24 hr after the last injection of zinc sulfate. BSO (2.5 mmol/kg) was administered s.c. to these mice at 4 hr prior to killing. Values are means ± SD for four mice.

*,†Significantly different from the corresponding untreated group: *$P < 0.01$, and †$P < 0.001$.

vided by Dr. A. Choo (Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Australia) [28], were of a mixed genetic background of 129 Ola and C57BL/6 strains. F1 hybrid mice were mated with C57BL/6 mice, and their offspring were back-crossed to C57BL/6 for three generations. MT-null ($MT^{-/-}$) mice and wild-type ($MT^{+/+}$) mice were obtained by mating of those heterozygous ($MT^{+/-}$) mice. We have found previously that *cis*-DDP nephrotoxicity is not significantly different between C57BL/6J mice and 129/Sv mice [22].

MT-null mice and wild-type mice were routinely bled in the vivarium of the National Institute for Environmental Studies (NIES). Microbiological and viral examinations were performed with regular quarantine procedures for more than a 1-year period, 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-hr light/dark cycle. They were maintained on standard laboratory chow and tap water *ad lib.*, and they received humane care throughout the experiment according to the guidelines of the NIES.

cis-DDP was supplied by the Nippon Kayaku Co., and the metal compounds and other chemicals were purchased from Wako Pure Chemical Industries.

Treatments

Eight-week-old female MT-null mice and wild-type mice (four mice for each treatment group) were given s.c. injections of zinc sulfate (200 μmol/kg) once a day for 2 days. These mice were given a single i.p. dose of *cis*-DDP (30 μmol/kg) at 24 hr after the last injection of zinc sulfate. L-BSO (2.5 mmol/kg) was administered s.c. to these mice 4 hr prior to a *cis*-DDP injection. To examine the renal toxicity of *cis*-DDP, blood and kidney were removed from each mouse under diethyl ether anesthesia 2 days after the injection of *cis*-DDP.

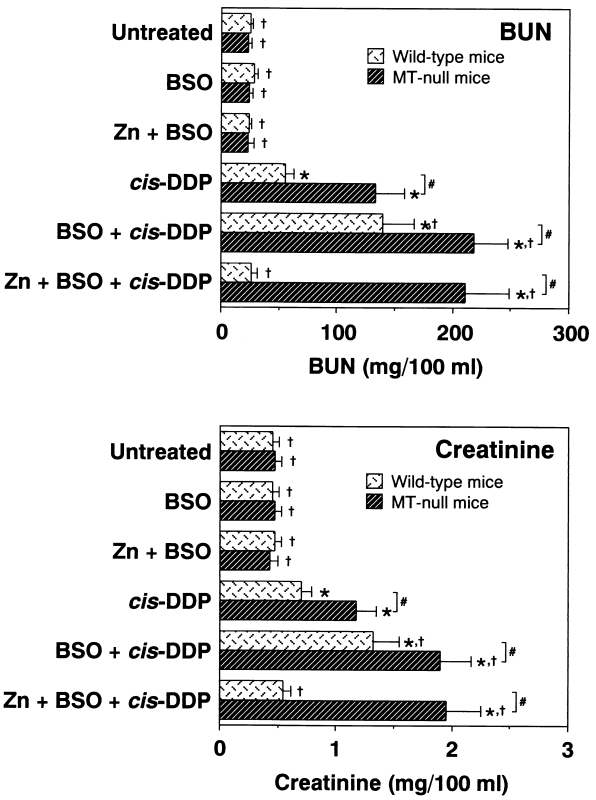


FIG. 1. Effect of pretreatment with BSO and zinc sulfate on renal toxicity caused by *cis*-DDP in MT-null mice and wild-type mice. BUN and creatinine were utilized as biomarkers for renal toxicity. Values are means ± SD for four mice. Key: (*) significantly different from the corresponding untreated group ($P < 0.05$); (†) significantly different from the corresponding *cis*-DDP-treated group ($P < 0.05$); and (#) significantly different from wild-type mice ($P < 0.05$).

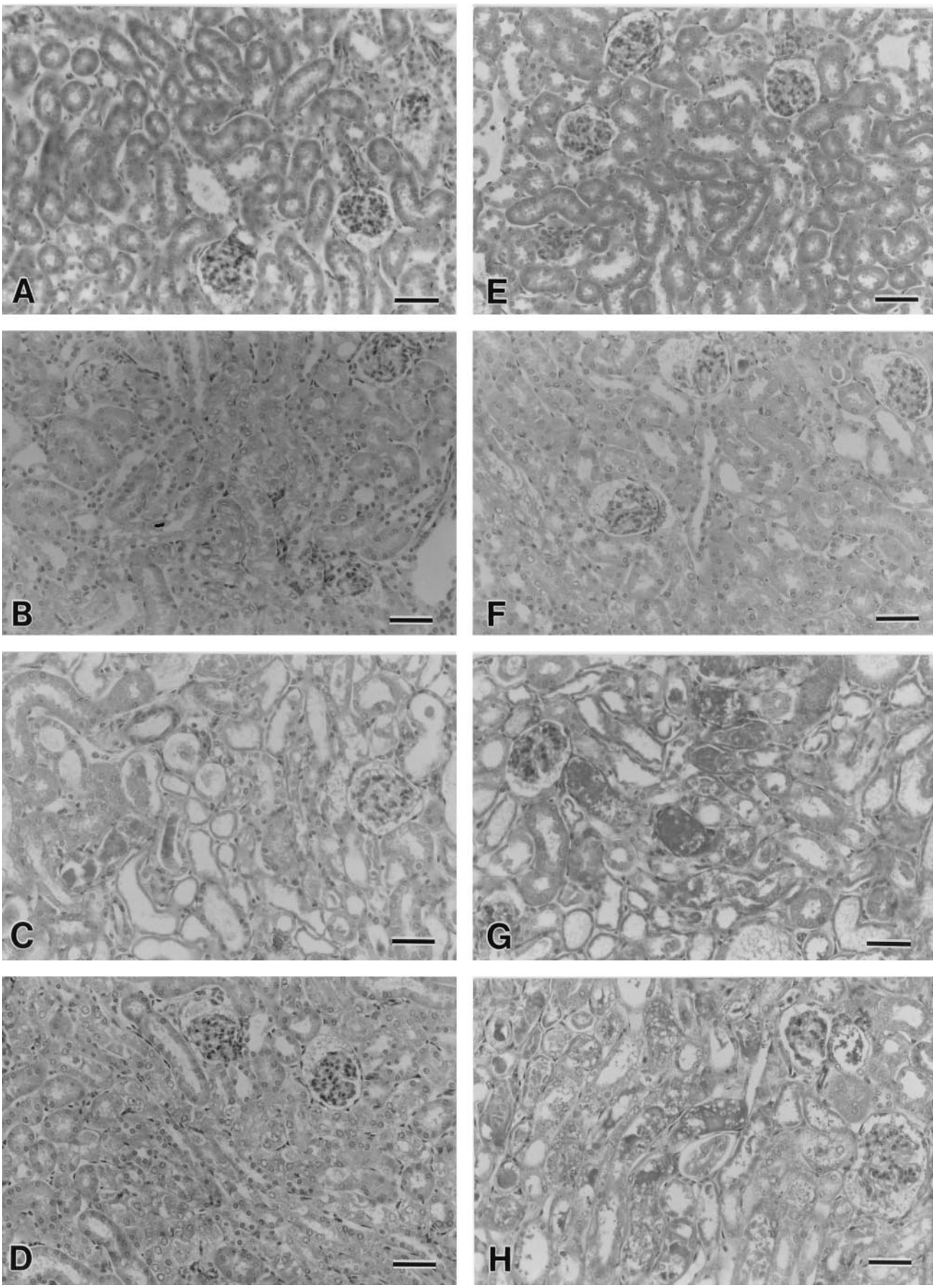
Histochemical Staining

For histochemical evaluation of nephrotoxicity, kidney tissues were fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin. Deparaffinized tissue sections of 5 μm in thickness were stained in hematoxylin-eosin.

Analysis

MT and GSH concentrations in the kidney were measured by radioimmunoassay [29] and Bioxytech GSH-400 Assay reagent (Oxis International, Inc.), respectively. BUN and

FIG. 2. Effect of pretreatment with BSO and zinc sulfate on histopathological changes in the renal cortex of *cis*-DDP-treated MT-null mice and wild-type mice. (A–D) wild-type mice; (E–H) MT-null mice. (A) untreated; (B) *cis*-DDP treated; (C) BSO and *cis*-DDP treated; (D) Zn, BSO, and *cis*-DDP treated; (E) untreated; (F) *cis*-DDP treated; (G) BSO and *cis*-DDP treated; and (H) Zn, BSO, and *cis*-DDP treated. Bar = 50 μm.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creatinine values in the serum were determined using the automatic dry-chemistry analyzer system (Spotchem SP-4410; Kyoto Daiichikagaku). The data were analyzed statistically by Student's *t*-test.

RESULTS

MT and GSH levels were determined in the kidneys of MT-null mice and wild-type mice treated with BSO and zinc sulfate (Table 1). The renal MT level in the wild-type mice was increased to approximately 2.5 and 30 times of the control level by BSO alone and by a combination of BSO and zinc sulfate, respectively. There were no detectable amounts of renal MT in untreated MT-null mice, and they could not be induced by either BSO treatment or a combination of BSO and zinc sulfate. On the other hand, the basal GSH concentration in the kidney was not significantly different between MT-null mice and wild-type mice. At 4 hr after the BSO treatment, the renal GSH levels in both the MT-null mice and the wild-type mice were decreased to approximately 20% of the control level. The decreased GSH levels in these mice were not affected by injection of the zinc sulfate.

As shown in Fig. 1, BUN and creatinine values in the MT-null mice and wild-type mice were increased significantly by *cis*-DDP injection, and the MT-null mice showed a high sensitivity to the renal toxicity of *cis*-DDP compared with the wild-type mice. The elevated levels of these indicators in both kinds of *cis*-DDP-injected mice were enhanced significantly by BSO pretreatment, but the enhanced renal toxicity was strongly apparent in the MT-null mice. In the wild-type mice treated with both BSO and *cis*-DDP, the high levels of these indicators were reduced markedly by preadministration of zinc sulfate, but this result did not occur in the MT-null mice.

Next, we examined histopathological changes in the renal cortex of MT-null mice and wild-type mice treated with *cis*-DDP (Fig. 2). Both MT-null mice and wild-type mice treated with *cis*-DDP showed a slight tubular damage such as a lesser degree of degenerative tubules (Fig. 2, B and F). BSO pretreatment and *cis*-DDP administration to the MT-null mice and wild-type mice resulted in marked morphological changes such as degeneration and necrosis in tubular cells and a urinary cast in tubular lumen, but the degree of tubular damage was found to be more extensive in the MT-null mice (Fig. 2, C and G). The administration of zinc sulfate remarkably prevented the BSO-enhanced tubular damage in the wild-type mice treated with *cis*-DDP (Fig. 2D), whereas MT-null mice pretreated with zinc sulfate before the *cis*-DDP and BSO treatment showed tubular damage as severe as that found in *cis*-DDP- and BSO-treated MT-null mice (Fig. 2, G and H).

DISCUSSION

The adverse effect of *cis*-DDP, a widely used antineoplastic drug, has been found to be associated with depletion of cellular thiol such as MT and GSH. The sensitivity of MT-null mice to *cis*-DDP nephrotoxicity has been found recently to be enhanced when compared with that of wild-type mice [22, 23]. In addition, fibroblasts derived from MT-null mouse embryos have been more sensitive to *cis*-DDP than MT-positive normal fibroblasts [30]. *cis*-DDP toxicity has been enhanced by BSO-mediated GSH depletion *in vivo* and *in vitro* [14, 15, 31, 32]. The present results are consistent with these earlier observations and show that kidney damage caused by *cis*-DDP was also enhanced by the depletion of MT and/or GSH. Moreover, we found that the renal toxicity caused by *cis*-DDP in the GSH-depleted MT-null mice was accelerated compared with that of the MT-null mice and the GSH-depleted wild-type mice. These results suggest that cellular MT and GSH act cooperatively as a detoxifying factor against severe kidney damage caused by *cis*-DDP.

Recently, a few reports have demonstrated a protective effect of zinc against the renal toxicity caused by cadmium-metallothionein (Cd-MT), the hepatotoxicity caused by carbon tetrachloride, and the insulin-dependent diabetes mellitus (IDDM) caused by streptozotocin in not only wild-type mice but also MT-null mice [33–35]. These results indicate that zinc-induced protection against Cd-MT nephrotoxicity, carbon tetrachloride hepatotoxicity, and streptozotocin IDDM is not due to MT. On the other hand, zinc pretreatment suppressed $CdCl_2$ hepatotoxicity and *cis*-DDP nephrotoxicity in the wild-type mice but not in the MT-null mice [22, 33]. These workers speculated that induction of MT by zinc pretreatment is capable of preventing adverse effects of these compounds. Thus, MT-null mice are an excellent model in which to evaluate whether the protection by metals such as zinc and bismuth against the toxicity of chemicals and metals is due to the preinduction of MT synthesis. Nakagawa and coworkers [24] showed that *cis*-DDP nephrotoxicity enhanced by BSO-mediated GSH depletion is prevented by zinc pretreatment. The present study clearly shows that induction of MT by zinc pretreatment is responsible for the *cis*-DDP-caused nephrotoxicity enhanced by GSH depletion, because severe kidney damage was prevented by zinc pretreatment in the GSH-depleted wild-type mice, but not in the GSH-depleted MT-null mice. This result suggests the hypothesis that MT can be an alternative detoxifying factor against renal toxicity caused by *cis*-DDP in cases where the kidney is deficient in GSH.

MT and GSH can act as free radical scavengers and prevent progression of lipid peroxidation [36, 37]. *cis*-DDP induces lipid peroxidation *in vitro* and *in vivo*, and this is reduced by antioxidants [8, 9]. Since platinum can be bound to the thiols in cysteine, GSH, and MT molecules, MT and GSH may prevent the *cis*-DDP-caused renal toxicity by trapping platinum and scavenging free radicals produced from *cis*-DDP.

Clinical cancer chemotherapy including *cis*-DDP often shows a large variation in the appearance and degree of nephrotoxicity in patients. Human MT and GSH levels

differ under various conditions and life-styles. GSH levels in tissues have a circadian rhythm [38] and are decreased by treatment with several anticancer drugs such as *cis*-DDP, adriamycin, and alkylating agents [18, 39, 40]. MT synthesis is easily induced by various metals, glucocorticoids, and many other factors including *cis*-DDP [21]. Thus, the variation of sensitivity to renal toxicity caused by *cis*-DDP may be due to alterations of endogenous renal MT and/or GSH levels.

In conclusion, the renal toxicity of *cis*-DDP was enhanced by MT or GSH depletion, but greatly accelerated by both MT and GSH depletion, suggesting that MT and GSH cooperatively play an important role in detoxification of severe kidney damage caused by *cis*-DDP. Alteration of the renal MT concentration probably negatively affects the development of *cis*-DDP nephrotoxicity caused by depletion of renal GSH, because the increased *cis*-DDP nephrotoxicity due to GSH depletion can be prevented by preinduction of MT and accelerated by MT-null mutation.

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