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Short communication

Metallothionein induction prevents toxic side effects of cisplatin and adriamycin used in combination

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Summary. The protective effects of metallothionein (MT) preinduction by bismuth subnitrate (BSN) against the renal toxicity of *cis*-diamminedichloroplatinum (*cis*-DDP), the cardiotoxicity of adriamycin (ADR), and the lethal and bone marrow toxicities of these drugs were observed in mice receiving *cis*-DDP and ADR simultaneously. Preinduction of MT biosynthesis by BSN, which is currently used as an antidiarrheal drug, did not affect the antitumor activities of the two drugs, suggesting that the beneficial effects of the preinduction of MT biosynthesis by BSN may be applicable for therapy involving *cis*-DDP and ADR, either alone or in combination.

Introduction

cis-Diamminedichloroplatinum (cisplatin: *cis*-DDP), a platinum coordination complex [14, 15], and adriamycin (ADR), an anthracycline antibiotic [5], are the most potent anticancer drugs, showing broad spectra as to activity against human neoplasms, particularly solid ones. Since the modes of actions of these two antitumor drugs are different, they are often used in combination for cancer chemotherapy. However, their clinical use is limited by their characteristic toxic side effects, which are mainly renal toxicity [9] and cardiotoxicity [8], respectively. We have recently found [11] that preinduction of the biosynthesis of metallothionein (MT) [19], a low-molecular-weight protein exhibiting a protective action against heavy metal toxicity, strongly depresses the lethal toxicity of *cis*-DDP. We report that the preinduction of MT biosynthesis is effective for reducing the toxicity of not only *cis*-DDP but also of ADR.

Materials and methods

ICR male mice ($n = 5$) were pretreated orally with bismuth subnitrate (BSN; 50 mg/kg) once a day for 5 consecutive days. *cis*-DDP and/or ADR were injected subcutaneously (s. c.) and intraperitoneally, respectively, 24 h after the last pretreatment with BSN. These mice were sacrificed 4 days after injection of the antitumor drugs. Blood urea nitrogen (BUN) values were measured spectrophotometrically as an indicator for renal toxicity using a BUN assay kit (Urea-N-test; Wako Pure Chemical Industries, Ltd., Tokyo). The number of leukocytes in the blood was counted using a Coulter counter as an indicator for bone marrow toxicity. Lipid peroxidation in the heart was determined by measuring the amounts of thiobarbiturate-reactive substances (TBA-RS) and conjugated dienes by methods of Ohkawa et al. [13] and Suryanarayana and Recknagel [17], respectively. The TBA-RS level was expressed as nmol malondialdehyde (MDA)/g heart. The conjugated diene level was expressed as absorbance at 232 nm.

Mice transplanted s.c. with colon adenocarcinoma 38 (colon 38) or Ehrlich tumor cells were used for evaluation of the effect of BSN pretreatment on the antitumor activity of ADR, as previously described [12].

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Results and discussion

To induce MT biosynthesis, BSN (50 mg/kg daily) was given orally to ICR male mice once a day for 5 consecutive days using a stomach tube [12]. Table 1 shows the lethal toxicities of *cis*-DDP and ADR injected either alone or in combination at 24 h after the last instillation of BSN. The mice pretreated with BSN showed significant tolerance not only to the individual drugs but also to their com-

Table 1. Effect of preinduction of MT biosynthesis by BSN on the survival rate of mice given *cis*-DDP and ADR simultaneously

Drug and dose		Survival rate (%)	
cis-DDP (μmol/kg)	ADR (μmol/kg)	Control mice	MT-preinduced mice
0	0	100	100
35	0	71	100
0	25	57	100
35	25	0	100
40	0	43	100
0	30	43	100
40	30	0	86
45	0	0	100
0	35	0	71
45	35	0	57

Biosynthesis of MT was induced by oral administration of BSN (50 mg/kg) to ICR male mice ($n = 7$) once a day for 5 consecutive days. *cis*-DDP and ADR were injected subcutaneously and intraperitoneally, respectively, 24 h after the last instillation of BSN. The survival rate was determined 20 days after injection of the antitumor drugs

bined administration (Table 1). The survival rate of mice given either *cis*-DDP (40 μmol/kg) or ADR (30 μmol/kg) was 43%. However, the lethal toxicity of either drug could be completely reversed by BSN pretreatment. The complete lethality observed in mice simultaneously injected with *cis*-DDP (40 μmol/kg) and ADR (30 μmol/kg) was also efficiently reversed by MT preinduction.

Figure 1 shows the effects of MT induction on the renal toxicity, cardiotoxicity, and bone marrow toxicity in mice following the injection of *cis*-DDP and/or ADR. Renal toxicity was observed in mice receiving *cis*-DDP alone or *cis*-DDP and ADR simultaneously. Coupled increases in the level of MDA and conjugated dienes in the hearts of mice was induced by ADR with or without *cis*-DDP. Moreover, a decrease in the total number of leukocytes was observed in the mice injected with ADR and/or *cis*-DDP. However, in the MT-preinduced mice, no significant change in the values for these indicators occurred on the injection of the antitumor drugs, even with concurrent injection of the two drugs. The antitumor activity of *cis*-DDP has been shown to be insensitive to BSN pretreatment [12]. In the present study, using the mice transplanted s.c. with colon 38 or Ehrlich tumor cells, we confirmed that the antitumor activity of ADR was not affected in BSN-pretreated mice, although the cardiotoxicity and bone marrow toxicity were efficiently depressed by preinduction of MT biosynthesis (data not shown). These results suggest that the preinduction of MT biosynthesis by BSN is useful for reduction of the toxic side effects of both *cis*-DDP and ADR, and that it may allow increases in the doses of the drugs, which in turn would lead to their higher efficacy in clinical use. Since the effective oral dosage of BSN (50 mg/kg daily) in the present experiments was not far from that commonly used (2 g/person daily), this treatment may soon be applicable in clinical treatment.

In mice inoculated s.c. with colon 38 cells, the MT levels in the tumor tissues did not change with the administration of BSN, although the cardiac and renal MT levels were significantly increased. Thus, the specific depressing effect of BSN pretreatment on the adverse effect of ADR may be explained by the inability of BSN to induce MT biosynthesis in tumor tissues.

Intracellular free radical generation has been considered as one of the possible mechanisms involved in the cytotoxic action of ADR [1, 4]. Furthermore, it has recently been reported that MT has a radical scavenging ability in vitro [16, 18]. We measured, therefore, the amount of superoxide radical generated in the postnuclear fraction of the heart with the addition of ADR in vitro by the nitroblue tetrazolium reduction method [3]. Compared with that

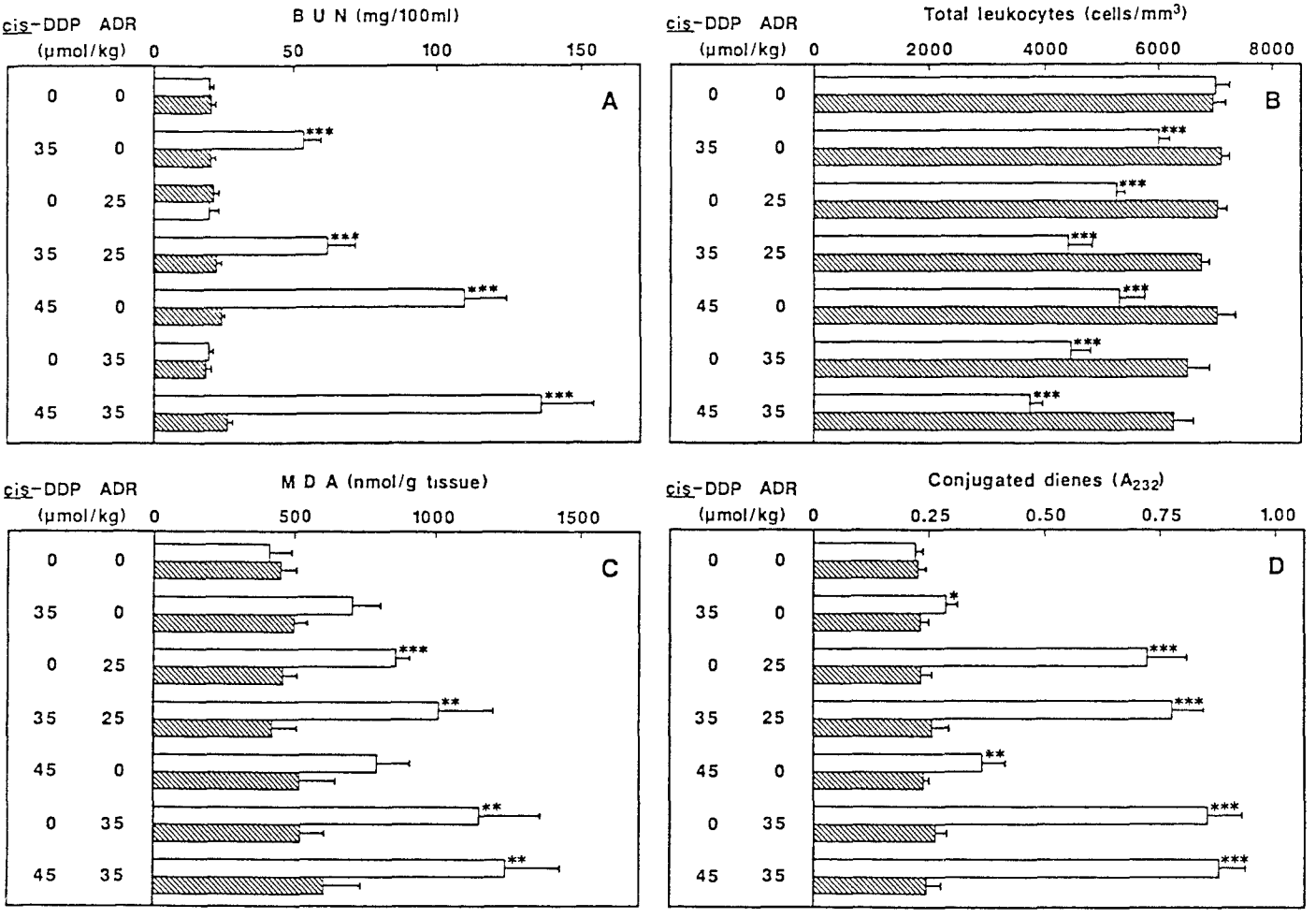

Fig. 1. Effect of preinduction of MT biosynthesis by BSN on the renal toxicity (*A*), bone marrow toxicity (*B*), and cardiotoxicity (*C, D*) of *cis*-DDP and/or ADR in mice. *, **, ***; Significantly different from the control (*$P<0.01$, **$P<0.005$, ***$P<0.001$). ☐, BSN-untreated mice; ▨, MT-preinduced mice

in control mice, the in vitro formation of superoxide radical was markedly depressed dose dependently by pretreatment of the mice with a bismuth compound (data not shown). Therefore, there is a possibility that MT depresses the cytotoxic action of ADR by interacting with oxygen radicals generated by the drug in the tissues. Recently, some reports have shown the possibility that MT endows mice [10] and cultured cells [2] with radioresistance. These experimental results indicate that MT induction is a promising technique for reducing the toxic side effects of antitumor drugs, not only for chemotherapy with metal-containing drugs, such as cisplatin, and free radical-forming drugs, such as ADR, bleomycin, and peplomycin, but also for therapy with irradiation, which has also been believed to exert damage in tissues by generating free radicals.

For cancer chemotherapy, a number of alkylating agents are also available as antitumor drugs. A protective effect of MT against the toxicities of some alkylating agents has also been reported [6, 7]. Thus, the induction of tissue MT biosynthesis, except in tumor tissues prior to challenging with antitumor drugs or irradiation, may be a generally useful tool for efficient management of the adverse side effects of therapeutic treatment without affecting their antitumor activities.

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