Prevention of Lethal and Renal Toxicity of cis-Diamminedichloroplatinum(II) by Induction of Metallothionein Synthesis without Compromising Its Antitumor Activity in Mice

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ABSTRACT

The participation of renal metallothionein (MT) in the toxicity and antitumor activity of cis-diamminedichloroplatinum(II) (cis-DDP) in male mice was examined. Pretreatment with MT in the kidney by the s.c. administration of bismuth compounds decreased the lethality and renal and gastrointestinal toxicity caused by a single s.c. injection of cis-DDP. In the present study a correlation between the protective effect of pretreatment with bismuth nitrate against cis-DDP toxicity and the preinduced MT levels in the kidney was observed. Bismuth nitrate pretreatment showed no effect on the antitumor activity of cis-DDP against several transplantable tumors, probably because it induces MT in the kidney but not in tumor tissues. The fact that p.o. pretreatment of bismuth subnitrate, an antidiarrheal drug, also depressed the lethal toxicity of cis-DDP is promising for its prompt application in medical attention. Thus, bismuth pretreatment allows higher doses of cis-DDP with no apparent toxicity, resulting in more efficient utilization of this anticancer drug.

INTRODUCTION

cis-DDP is a potent antitumor agent containing the heavy metal, platinum (1-4). However, its application in large doses is limited by several toxic side effects, of which its nephrotoxicity is well known as the most common dose-limiting factor (5, 6). Bakka et al. (7) reported that cultured cells with a high content of MT were resistant to cis-DDP toxicity. MT is an inducible protein of low molecular weight synthesized in various mammalian tissues in response to heavy metals including zinc, copper, cadmium, and mercury, and it has a high affinity for these metals (8, 9). A physiological role for MT in heavy metal detoxification has been postulated (10, 11). Some investigators observed the binding of platinum to MT in the liver and kidney of cis-DDP-treated rats (12-14), and cis-DDP itself has an ability to induce MT in the liver but not in the kidney, a primary target of cis-DDP toxicity (15). The purpose of this investigation was to determine the effect of preinduction of renal MT on the toxicity and antitumor activity of cis-DDP. In this study, bismuth compounds were used as MT inducers, because bismuth is a potent inducer for accelerating MT synthesis specifically in the kidney (16, 17).

MATERIALS AND METHODS

Animals. Male ICR mice were purchased from Charles River Japan, Inc., Atsugi, Japan. BALB/c, C57BL/6, DBA/2, CDF2, and B6D2F, male mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan.

Chemicals. cis-DDP was kindly supplied by Nippon Kayaku Co., Ltd., Tokyo, Japan. BSN was purchased from Iwaki Co., Ltd., Tokyo, Japan. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. cis-DDP and bismuth compounds were dissolved in saline and distilled water, respectively, prior to use.

Tumors. Ehrlich carcinoma was supplied by Nippon Kayaku Co., Ltd. and passaged by i.p. transplantation in male ICR mice. P388 leukemia and colon adenocarcinomas 26 and 38 were kindly supplied by Dr. T. Tsuruo, Japanese Foundation for Cancer Research, Tokyo, Japan. P388 was passaged by i.p. transplantation in male DBA/2 mice. Colon 26 and 38 were maintained by s.c. transplantation in the backs of male BALB/c mice and C57BL/6 mice, respectively. The viability of tumor cells was tested by trypan blue exclusion.

Treatment with Drugs. BN was s.c. administered once a day for 1, 2, or 3 days. p.o. administration of BN and BSN were performed once a day for 5 days by stomach tube. These mice were s.c. given a single dose of cis-DDP (35 μmol/kg) at 24 h after the last administration of bismuth compound.

Evaluation of Antitumor Activity. The effect of BN pretreatment on the antitumor activity of cis-DDP against ascites tumors was examined by using P388 leukemia-inoculated mice. On day 0, P388 (10^6 cells) was injected i.p. to B6D2F, mice aged 8 weeks. These mice received BN (30 μmol/kg/day, s.c.) on days 1 and 2, and a single dose of cis-DDP (25, 30, 35, or 45 μmol/kg, s.c.) on day 3. The antitumor activity was evaluated by the mean survival time of a group of mice.

Mice given transplants of Ehrlich carcinoma, colon 26, or 38 were used for evaluation of the effect of BN pretreatment on antitumor activity of cis-DDP against solid tumors. Ehrlich carcinoma (2 x 10^6 cells) was injected s.c. in the backs of ICR mice on day 0. Colon 26 tumor lump from BALB/c mice or colon 38 tumor lump from C57BL/6 mice was minced in Hanks’ balanced salt solution to prepare a suspension (33%, w/v). Each suspension of colon 26 or 38 (about 2 x 10^6 cells/mouse) was inoculated s.c. into the backs of CDF2 mice (8 weeks old) or B6D2F mice (8 weeks old) on day 0, respectively. Two s.c. doses of BN were administered at 24-h intervals on days 1 and 2, and cis-DDP was administered s.c. in the abdomen of the mice on day 3. Antitumor activity was evaluated by tumor growth on days 8 and 15 (5 and 12 days after cis-DDP administration) and BUN values were determined on day 8. p.o. administration of BSN to the ICR mice inoculated s.c. with Ehrlich carcinoma on day 0 as described above was performed once a day from days 1 to 5, and cis-DDP was administered s.c. to the mice on day 6. Tumor weight was measured after removal of a whole tumor lump from each mouse under ether anesthesia on the indicated days.

Determination of MT. MT levels in the kidney and liver of the mice at 24 h after the last administration of the bismuth compound (without cis-DDP injection) were determined by the ^203Hg-binding assay (18, 19) as modified by the present authors (20). Two ml of tissue homogenate (5%, w/v) in 0.1 M tri-HCl (pH 7.6) was incubated with 10 μl of diethylnalate at 25°C for 15 min. After the addition of 50 μl of 10 mM cadmium chloride, particles and high-molecular weight proteins were removed by heating at 100°C for 3 min. The aqueous fraction was added with excess ^203HgCl (100 nmol and 0.2 μCi) to saturate the metal binding sites of MT by ^203Hg. Non-MT-bound ^203Hg was removed by the addition of ovalbumin (1 μmol) followed by acidification with 100 μl of 100% trichloroacetic acid solution. Then the radioactivity of 203Hg bound to MT was measured, and the content was expressed as nmol mercury bound to MT. It was confirmed that mercury completely replaced zinc and bismuth bound to MT, and the ^203Hg finally determined in the trichloroacetic acid-soluble fraction specifically bound to...
MT. The concentration of MT in the tumor was also determined. Mice were inoculated s.c. with colon 38 on day 0. BN (30 μmol/kg/day, s.c.) was administered on days 14 and 15, and then MT content in the tumors was determined on day 16.

Other Methods. BUN values were measured spectrophotometrically using a BUN assay kit (Urea-N-test; Wako). The number of leukocytes was counted using a Coulter Counter (ZBI type).

RESULTS

Effect of BN Treatment on cis-DDP Toxicity. The effect of s.c. preadministration of BN at various administration schedules on the toxicity of cis-DDP (35 μmol/kg, s.c.) in mice was examined. cis-DDP was administered s.c. 24 h after the last injection of BN. S.c. preadministration of BN apparently reduced the acute lethal toxicity of cis-DDP (Table 1). Some of the administration schedules completely eliminated the cis-DDP lethal toxicity. The renal MT content of the mice at 24 h after the last injection of BN was significantly increased in comparison with that of the control mice. A distinctive correlation was found between the protective effects of BN (rate of mice surviving at 15 days after cis-DDP injection; see Table 1) and the preinduced MT levels in the kidneys of mice given BN (Fig. 1).

To prevent the lethal toxicity of cis-DDP, administration of BN prior to cis-DDP injection was essential (Table 2). The preadministration of BN (30 μmol/kg/day, s.c.) once a day for 2 days prior to cis-DDP injection efficiently depressed not only the lethal toxicity but also the incidence of diarrhea (Table 2) and the increase in BUN (Fig. 2) caused by cis-DDP.

Effect of BN Pretreatment on Antitumor Activity of cis-DDP. Fig. 3 shows the effect of pretreatment of mice with two s.c. doses of BN at 24-h intervals on the antitumor activity of cis-DDP in mice inoculated i.p. with P388 leukemia cells. The BN pretreatment did not affect the antitumor activity of nonlethal doses (20 or 25 μmol/kg) of cis-DDP. cis-DDP alone at a dosage of 35 or 45 μmol/kg was very toxic and all the mice receiving these doses of cis-DDP died within 8 days after the injection without a visual increase in the volume of abdominal ascites. Pretreatment with BN completely depressed the lethal toxicity of cis-DDP and efficiently prolonged the survival time of the P388-bearing mice receiving cis-DDP (35 or 45 μmol/kg). These valuable effects of BN pretreatment were also observed in solid tumor-bearing mice. As shown in Table 3, BN pretreatment of the solid tumor-bearing mice efficiently depressed the lethal and renal toxicity of cis-DDP without compromising its antitumor activity and allowed the administration of a relatively high dose of cis-DDP.

In order to examine the effect of BN on MT levels in tumor,

<table>
<thead>
<tr>
<th>Pretreatment dose of BN (μmol/kg/day)</th>
<th>No. of survivors after cis-DDP injection, at day</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0 4 5 6 7 8 10 15</td>
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<tr>
<td>40 × 1</td>
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Table 1 Effect of BN pretreatment on lethal toxicity of cis-DDP in mice

Mice (ICR) were pretreated with a s.c. dose of BN once a day for 1, 2, or 3 days. cis-DDP (35 μmol/kg, s.c.) was administered to the mice 24 h after the last injection of BN.

Fig. 1. Relationship between the protective effect of BN pretreatment against cis-DDP toxicity (survival rate of mice 20 days after cis-DDP injection; see Table 1) and the MT levels (each value of MT content is the mean ± SD of 5 mice) in kidney of mice at 24 h after the last injection of BN.

Fig. 2. Effect of BN pretreatment on BUN values in mice receiving m-DDP. Mice (ICR) were pretreated with two s.c. doses of BN (30 μmol/kg) at 24-h intervals and were then given cis-DDP (35 μmol/kg, s.c.) 24 h after the last injection of BN (D), cis-DDP alone at a dose of 30 μmol/kg (■), or 35 μmol/kg (□) without BN pretreatment. All of the mice that received cis-DDP alone at a dose of 35 μmol/kg died within 6 days. D, control mice. Values, mean ± SD (bars) of 4 mice.

Fig. 3. Effect of BN pretreatment on antitumor activity of cis-DDP in mice inoculated i.p. with P388 leukemia cells. Mice (B6DF1) were inoculated i.p. with P388 leukemia on day 0. They were pretreated with two s.c. doses (30 μmol/kg) of BN (○) or saline (□) at 24-h intervals on days 1 and 2. cis-DDP was administered s.c. on day 3. Values, mean ± SD (bars) of 10 mice.

BN (30 μmol/kg/day) was administered to solid tumor (colon 38)-bearing mice once a day for 2 days. The MT concentration (nmol mercury bound) in the tumors of mice at 24 h after the last injection of BN [78.2 ± 10.7 (SD)] did not exceed that of BN-untreated mice (61.7 ± 22.3). In the kidneys of these mice, a 7-fold increase in MT concentration was observed.

Effect of p.o. Preadministration of BSN on the Lethal Toxicity of cis-DDP. Because BSN and some other bismuth compounds
compared with the control, and completely depressed the lethal result. A 6-fold increase in the renal MT level when treatment with BSN or BN at a dose of over 50 mg/kg/day resulted in a 24-h increase in the renal MT level when compared with the control, and completely depressed the lethal toxicity of cis-DDP (Table 4). All the mice receiving cis-DDP showed a significant loss in body weight during the first six days after injection, but a recovery was observed in BN- and BSN-pretreated mice. Neither BN nor BSN caused any loss in body weight of mice, nor were there any other visible toxic signs during the experiment. In separate experiments, BSN showed a significant loss in body weight during the first six days after injection, but a recovery was observed in BN- and BSN-pretreated mice. Neither BN nor BSN caused any loss in body weight of mice, nor were there any other visible toxic signs during the experiment. 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DISCUSSION

Although a well-defined physiological function of MT has not yet been established, many studies on MT have emphasized its role in the homeostasis of essential metals (21) and detoxification of nonessential heavy metals (10, 11). The present results demonstrate clearly the protective effect of bismuth, a potent and specific inducer of renal MT, against the lethal and renal toxicity of cis-DDP in mice inoculated s.c. with Ehrlich tumor cells (Fig. 3; Table 3). This property of bismuth compounds makes them ideal for clinical application as protective agents against the toxic side effects of cis-DDP. Moreover, it is expected that bismuth pretreatment will allow the administration of relatively high doses of cis-DDP, making it a more useful drug. BSN and some other bismuth compounds have been used as antidiarrheal drugs administered p.o. Since the effective p.o. dosage of BSN (33.3 mg/kg/day) to reduce the lethal toxicity of cis-DDP is not far from that commonly used for humans (2 g/person), this treatment may soon be applicable in clinical use. Since the maximal induction of renal MT was not shown), BSN should be administered at 12- to 24-h intervals.

Several attempts have so far been made to reduce cis-DDP toxicity (26, 28, 30-38). Although effective diuresis regimens are generally accepted at present (39), hydration takes a very long time and burdens the patient with a painful load. Bismuth pretreatment seems to be simple and effective enough to allow higher doses of cis-DDP. Additionally, combining cis-DDP with other regimens which have so far been reported may provide more successful clinical utilization of this anticancer drug.

REFERENCES

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DETOXICATION OF cis-DDP BY RENAL METALLOTHIONEIN


Prevention of Lethal and Renal Toxicity of \textit{cis}-Diamminedichloroplatinum(II) by Induction of Metallothionein Synthesis without Compromising Its Antitumor Activity in Mice

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