Modulation of Both Cisplatin Nephrotoxicity and Drug Resistance in Murine Bladder Tumor by Controlling Metallothionein Synthesis

Masahiko Satoh, Debra M. Kloth, Salam A. Kadhim, Joseph L. Chin, Akira Naganuma, Nobumasa Imura, and M. George Cherian

Department of Public Health, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, Japan; M. S. A. N., N. J./. and Department of Pathology, Health Sciences Center [M. S. D. M. K., M. G. C.] and Department of Surgery, University Hospital [S. A. K., J. L. C.], University of Western Ontario, London, Ontario, N6A 5C1, Canada

ABSTRACT

The role of metallothionein (MT) in cisplatin (cis-DDP) resistance and renal toxicity was investigated in C3H mice inoculated with mouse bladder tumor (MBT-2). C3H mice were inoculated s.c. with 1 x 10⁶ MBT-2 cells/mouse on day 0. Mice were given injections of propargylglycine (PPG) (500 μmol/kg s.c.) once a day for 3 days from day 7 to day 9 and with ZnSO₄ (200 μmol/kg s.c.) once a day for 2 days from day 8 to day 9. cis-DDP (50 μmol/kg i.p.) was administered 10 days after MBT-2 cell inoculation. Since MT contents in the tumor and kidneys were significantly increased by administration of ZnSO₄, both the antitumor activity of cis-DDP and its renal toxicity were reduced. However, coadministration of PPG reduced MT induction in tumor without affecting the level of renal MT. As a result, PPG could clearly overcome the MT-mediated cis-DDP resistance of tumors without compromising the protective effect exerted by renal MT on nephrotoxicity of the drug. It was suggested, therefore, that PPG may be a promising adjunct in cancer chemotherapy to overcome the drug resistance of tumors caused by the elevated level of MT.

INTRODUCTION

cis-DDP, a coordination complex of platinum, is one of the most effective antineoplastic agents with a wide spectrum of therapeutic activity against certain human neoplasms such as tumors of the testis, ovaries, bladder, head, and neck (1–3). However, the clinical use of cis-DDP is limited by its dose-dependent side effects such as the severe nephrotoxicity (4) and development of drug resistance in certain tumors. It has been reported that induction of MT synthesis in animals by administration of heavy metals such as zinc and bismuth can provide protection against the toxic effects of cis-DDP (5, 6). MT is a cysteine-rich protein of low molecular weight and shows high affinity for metals such as zinc, copper, cadmium, mercury, and platinum (7). One of the proposed biological functions of MT is the detoxication of heavy metals.

Although the mechanisms involved in the protection of cis-DDP nephrotoxicity by MT induction are still unclear, the sequestration of cis-DDP or its metabolites by MT molecules and the antioxidant properties of MT have been proposed as possible mechanisms of detoxication (8, 9). Naganuma et al. have shown that p.o. administration of bismuth subnitrate resulted in a substantial reduction in cis-DDP-induced renal toxicity without compromising its antitumor activity (10). Moreover, a few clinical trials have suggested that pretreatment with bismuth compounds was effective in the reduction of renal toxicity caused by cis-DDP (11, 12).

In addition, the therapeutic efficacy of cis-DDP is sometimes decreased during chemotherapy for a relatively long period by development of drug resistance, often resulting in failure of cancer treatment. A variety of mechanisms of cis-DDP resistance have been described (13), e.g., decreased accumulation of drug (14), increased detoxification by thiol containing scavenger molecules such as GSH (15), and increased repair of DNA damage (16). There is some evidence that overexpression of the MT gene in tumors may associate with increased cis-DDP resistance. Cells containing high MT and demonstrating resistance to cadmium also show cross-resistance to cis-DDP (17–19). Recent studies on tumor cells transfected with the human MT-IIA gene showed resistance to cis-DDP as well as other alkylating agents (20). Furthermore, both the MT content and MT mRNA levels were found to correlate with resistance in human small cell lung cancer cell lines (21). Several investigators have reported that elevation of MT level in tumor leads to resistance against cis-DDP in tumor-bearing mice (6, 22). Thus, both in vitro and in vivo studies suggest that MT is an important factor in cis-DDP resistance in tumor cells of different origins.

To further elucidate the role of MT in cis-DDP resistance and renal dysfunction, in the present study, we have examined methods to inhibit tumor MT synthesis. PPG, a specific inhibitor of the cystathionine pathway, was injected into mice bearing murine bladder tumor (MBT-2) to decrease the intracellular pool of free cysteine, thereby decreasing MT synthesis (23). The antitumor activity and nephrotoxicity of cis-DDP were investigated in these mice.

MATERIALS AND METHODS

Bladder Tumor Model. MBT-2, a transitional cell carcinoma of the bladder [originally derived by Soloway (24)], was maintained in vivo as a solid s.c. growing tumor by weekly serial passaging in female C3H mice as described elsewhere (25). Single cell suspensions were prepared by enzymatic digestion of the minced solid tumor with a mixture of collagenase type II (1 mg/ml), proteinase K (0.01 mg/ml), and DNase (0.01 mg/ml) (all from Sigma Chemical Co., St. Louis, MO) for 20 min at 37°C with stirring. Tumor cell viability was determined by trypan blue exclusion. These cells were used for inoculation.

Animals and Treatments. Five-week-old female C3H mice (Charles River, Canada) were fed with Purina mouse chow and water ad libitum and maintained under standard laboratory conditions for 5 days before the inoculation of MBT-2 cells. A group of mice were inoculated s.c. with 1 x 10⁶ MBT-2 cells/mouse into the left flank region of the abdomen. Approximately 90–95% of all the inoculated animals developed s.c. tumors 8 days after inoculation. All tumors represented solid mass with minimum or no necrosis at the early stage of growth. Eight days after MBT-2 inoculation, tumor-bearing mice were randomized into control and experimental groups with four mice/group. The treatment schedule is shown in Fig. 1. Briefly, on day 8 a group of mice were given s.c. injections of either ZnSO₄ (200 μmol/kg) or saline into the right flank region of the abdomen once a day for 2 days. A group of ZnSO₄ or saline-injected mice were given PPG (Sigma) at a dose of 500 μmol/kg s.c. into the backs once a day for 3 days from day 7 to day 9 (before and along with ZnSO₄ injection) and with one dose of cis-DDP (50 μmol/kg i.p.) on day 10.

The antitumor activity and renal toxicity were determined on day 14 (4 days after the injection of cis-DDP). MT, GSH, zinc and copper levels in tissues were measured on day 14 (at the time of cis-DDP injection).

Antitumor Activity and Renal Toxicity. The antitumor activity was evaluated by tumor weight. The renal toxicity was evaluated by BUN. BUN values were measured on day 14 (at the time of cis-DDP injection).

Received 4/27/92; accepted 2/11/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Grant-in-Aid 03258238 for Special Project Research, Cancer-Bioscience, from the Ministry of Education, Science, and Culture, Japan.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: cis-DDP, cisplatin; MT, metallothionein; GSH, glutathione; PPG, propargylglycine; MBT-2, N4(4-(5-nitro-2-furyl)-2-thiazolyl)formamidine induced mouse bladder tumor; BUN, blood urea nitrogen.

Downloaded from cancertres.aacjrournals.org on April 19, 2017. © 1993 American Association for Cancer Research.
were measured spectrophotometrically using a urea nitrogen assay kit (Sigma) as described by Marsh et al. (26).

Analysis. MT contents in tissues were measured by the silver-hem saturation method using nonradioactive silver (27). Zinc and copper contents in tissues were determined by flame atomic absorption spectrosopy (Varian Spectra-30; Varian Canada, Georgetown, Ontario, Canada) after digestion with concentrated HNO₃. GSH contents in the tissues were measured by a method using the production of a chromophore resulting from the reaction of GSH with 5,5-dithiobis-2-nitrobenzoic acid (28).

Statistical Calculations. The data were analyzed by Student's *t* test and one way analysis of variance.

RESULTS

Effect of PPG on MT, GSH, and Metal Levels in Tissues of Tumor-bearing Mice. To examine the effect of PPG on MT and GSH levels in normal and tumor tissues, bladder tumor-bearing mice were treated s.c. with PPG. Fig. 2 shows the effect of PPG on GSH concentrations in the liver, kidney and tumor of mice with or without ZnSO₄ treatment. Although PPG has been known as a potent inhibitor of cystathionine pathway for synthesis of cysteine which is an important sulfhydryl source for GSH synthesis, GSH levels in the liver, kidney, and tumor of mice were not significantly altered by PPG and/or ZnSO₄ treatment. MT contents in tissues of tumor-bearing mice treated with ZnSO₄ and/or PPG are shown in Fig. 3. MT concentrations in the tumor, kidney, and liver of ZnSO₄-treated mice significantly (*P* < 0.001) increased as compared to those of untreated mice. In combination with PPG, however, the extent of MT induction by ZnSO₄ was significantly (*P* < 0.001) reduced to control level in the tumor and was significantly (*P* < 0.001) decreased in the liver but was unchanged in the kidney. Although zinc concentrations in these tissues were also increased by administration of ZnSO₄, the concurrent administration of PPG significantly reduced the extent of increase in zinc contents in the tumor and liver without affecting the elevated level of renal zinc (Table 1). The tissue MT concentrations seem to be correlated with zinc concentration. Copper contents in the tissues were unchanged by treatment with ZnSO₄ and/or PPG (data not shown).

Effects of PPG on Antitumor Activity and Renal Toxicity of cis-DDP. The effect of altered MT synthesis on antitumor activity of cis-DDP in tumor-bearing mice is shown in Fig. 4. Although the tumor weight of mice treated with cis-DDP was approximately 20% of untreated mice, its antitumor activity was significantly depressed by preinduction of MT synthesis by ZnSO₄. The inhibitory effect of zinc-induced MT on the antitumor activity of cis-DDP was prevented by PPG injection. PPG treatment alone had little effect on the antitumor activity of cis-DDP (Fig. 4). Nephrotoxicity has been identified as the most common and important side effect of cis-DDP treatment. Thus, BUN values were determined to evaluate the renal toxicity in these experiments. cis-DDP injection increased BUN values significantly (Fig. 5). Preinduction of MT by ZnSO₄ injection prevented the renal toxicity of cis-DDP, as shown by the decrease in BUN values to the control level. This protective effect of ZnSO₄-pretreatment on renal toxicity of cis-DDP was not compromised by treatment with PPG. The increase in BUN value caused by cis-DDP treatment was not affected by injection of PPG alone.

DISCUSSION

Several *in vitro* studies have shown that elevation of MT levels in certain cultured cells can result in resistance to cis-DDP (17-21). Endresen et al. (22) reported that cadmium-resistant mouse fibroblasts cells with high MT content transplanted to nude mouse showed resistance to cis-DDP *in vivo*. Furthermore, we have previously shown that pretreatment of tumor-bearing mice with zinc diminished the antitumor activity of cis-DDP, because zinc can induce MT synthesis

Fig. 2. Effect of PPG on GSH contents in tissues of tumor-bearing mice with or without ZnSO₄ treatment. The values are mean ± SD (bars) for four mice. ■ - ZnSO₄. ■ + ZnSO₄.
OVERCOMING OF CISPLATIN RESISTANCE

Fig. 3. Effect of PPG on MT contents in tissues of tumor-bearing mice with or without ZnSO4 treatment. The values are mean ± SD (bars) for four mice. ■ = ZnSO4, □ + ZnSO4.

*, P < 0.001.

Table 1 Effect of PPG on zinc contents in tissues of tumor-bearing mice with or without ZnSO4 treatment

<table>
<thead>
<tr>
<th></th>
<th>Tumor (µg/g tissue)</th>
<th>Kidney (µg/g tissue)</th>
<th>Liver (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.5 ± 1.5*</td>
<td>14.9 ± 2.2</td>
<td>30.5 ± 1.0</td>
</tr>
<tr>
<td>ZnSO4</td>
<td>20.0 ± 3.4*</td>
<td>32.1 ± 2.4</td>
<td>78.1 ± 16.2</td>
</tr>
<tr>
<td>PPG</td>
<td>11.5 ± 1.9</td>
<td>14.7 ± 2.0</td>
<td>31.7 ± 1.5</td>
</tr>
<tr>
<td>ZnSO4 + PPG</td>
<td>15.0 ± 1.4*</td>
<td>29.4 ± 3.0</td>
<td>46.3 ± 2.6</td>
</tr>
</tbody>
</table>

* Mean ± SD for four mice.

* and ** Significantly different from control (P < 0.01, **P < 0.001).

Fig. 4. Antitumor activity of cis-DDP in tumor-bearing mice treated with ZnSO4 and/or PPG. The values are mean ± SD (bars) for four mice. *, significantly different from control (P < 0.001).

not only in organs but also in the tumor tissue (6). Thus, MT may be one of the important cellular factors involved in acquiring cis-DDP resistance. However, attempts to overcome the cis-DDP resistance due to elevation of MT level in tumors have not been reported. In the present study, we found that PPG injection could diminish cis-DDP resistance acquired by an increase in MT levels. The effect of PPG on cis-DDP resistance could be explained by specific suppression of induced MT synthesis in the tumor by decreasing intracellular cysteine pools, although we have no direct proof of this since cysteine levels were not measured (Fig. 4).

PPG has been known as an irreversible inhibitor of γ-cystathionase (29). In rat hepatocytes, it has been shown that methionine was utilized as a sulfhydryl source for synthesis of MT and GSH via the cystathionine pathway (30, 31). Gallant and Cherian (23) have shown that inhibition of the cystathionine pathway in newborn rats with PPG decreased hepatic levels of both MT and GSH. However, the cystathionine pathway is not considered as a major source of cysteine for GSH synthesis in the kidney cells, because addition of methionine into the medium could not resume GSH synthesis in GSH-depleted kidney cells (32). Bannai and Tateishi (33) demonstrated that liver cells, unlike kidney cells, actively synthesize cysteine from methionine and serine via the cystathionine pathway and can utilize it for GSH synthesis. In the present study, PPG injection significantly inhibited the zinc-induced MT synthesis in both the liver and the tumor but not in the kidney, although GSH levels in tissues were not affected by PPG treatment. The mechanisms involved in the specific inhibition of MT synthesis in liver and tumor are still unclear. However, it is possible that the dose of PPG (500 µmol/kg) used in the present experiment was just enough to reduce the intracellular cysteine level to inhibit the induced synthesis of MT in liver and tumor tissue. Since GSH levels are not decreased in tissues, it may also suggest that the cysteine generated from the cystathionine pathway can be preferentially used for induced synthesis of MT. A recent study in adult rat showed no change in hepatic GSH levels after inhibition of cystathionase activity (34). A dose of 500 µmol/kg in C3H mice did not show any toxicity, but a similar dose was toxic to the rats, as shown by marked reduction in body weight.

The results suggest that the MBT-2 cells, a transplantable tumor, may synthesize intracellular cysteine mainly from methionine, similar to hepatic cells. However, more work is needed to confirm the presence of the cystathionine pathway in various animal and human tumor cells.
On the other hand, PPG did not alter the protective effect of zinc on the renal toxicity of cis-DDP (Fig. 5). It has recently been shown that pretreatment with bismuth compounds reduced markedly the toxicity of various anticancer drugs such as cis-DDP (10, 35, 36), Adriamycin (36–38) and bleomycin (6), tumor necrosis factor (39), and γ-irradiation (40) without compromising their antitumor activities. Bismuth compounds induce specifically MT synthesis in normal tissues such as kidney, heart, and bone marrow but not in tumors (10, 35, 37). In the present study, PPG injection inhibited the induction of MT synthesis by zinc in the tumor without affecting the induction of renal MT (Fig. 3). Thus, in the chemotherapy of patients bearing cis-DDP-resistant tumor, the combination of a bismuth compound and PPG may be useful to overcome the resistance and to protect its renal toxicity.

REFERENCES


Modulation of Both Cisplatin Nephrotoxicity and Drug Resistance in Murine Bladder Tumor by Controlling Metallothionein Synthesis

Masahiko Satoh, Debra M. Kloth, Salam A. Kadhim, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/8/1829

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.