Prevention of Carcinogenicity of Anticancer Drugs by Metallothionein Induction

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Abstract

We examined the efficacy of metallothionein induction in the prevention of the carcinogenic action of cis-platinum and melphalan administered repeatedly to mice over a relatively long period. The increased pulmonary metalthionein induced by bismuth or zinc compounds during the period of chemotherapy with cis-platinum or melphalan protected the mice from carcinogenesis of these drugs in the lung. These results suggested the efficacy of metallothionein inducers in suppression of carcinogenicity considered as a secondary effect of anticancer agents in cancer chemotherapy.

Introduction

Chemo- and radiotherapies have made remarkable progress with the development of new drugs and establishment of protocols with high efficacy in cancer treatment. However, secondary carcinogenesis is a major problem in long-term chemo- and radiotherapies, because almost all anticancer agents and γ-rays exert carcinogenicity (1). It has recently been reported that the acute toxic side effects of anticancer agents (2-5) and γ-irradiation (6) were prevented by increasing the amount of MT* in the target tissues of such toxicity. MT is a metal-binding protein of low molecular weight and its synthesis is induced by heavy metals and many other factors (7). It was reported that carcinogenesis in mice treated with 9,10-dimethyl-1,2-benzanthracene (8) and 3-methylcholanthrene (9) was inhibited by pretreatment with zinc, which is an inducer of MT, although the role of MT was not discussed at all by these authors. These findings prompted us to investigate the preventive effects of MT induction on the carcinogenic action of anticancer agents in appropriate tissues.

Materials and Methods

Animals and Chemicals. Five-week-old female A/J mice were purchased from Japan SLC, Hamamatsu, Japan. Three or four mice were housed in a cage under specific-pathogen-free conditions and were given free access to food and tap water. cis-DDP was kindly supplied by Nippon Kayaku Co., Ltd., Tokyo, Japan. L-PAM was purchased from Sigma Chemical Company, St. Louis, MO. Metal compounds and other chemicals were purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

Treatment and Analysis. One set of 10-13 female A/J mice were given i.p. injections of cis-DDP (2.0 μmol/kg/day for 24 days) or L-PAM (4.0 μmol/kg/day for 24 days) every other day for 3 days in a week. The mice were also given s.c. injections of either ZnCl2 (40 μmol/kg/day for 24 days) or Bi(NO3)3 (50 μmol/kg/day for 24 days) 24 h before administration of each anticancer agent. The schedule was repeated 8 times for 8 weeks. The number of tumors in the lung was determined 16 weeks after the last administration of anticancer agents. MT contents in the tissues were determined 4, 8, 16, and 24 weeks after the first injection of drugs. MT levels were measured by our modification (2) of the 203Hg-binding assay (10) and expressed as nmol 203Hg bound to MT. Data were analyzed by χ2 test or Student's t test.

Results and Discussion

A/J mice pretreated with a bismuth or zinc compound as an MT inducer were given cis-DDP or L-PAM, which is known to induce lung tumors in this strain of mice within 4 months after treatment (11, 12), and the number of nodules formed in the lung was counted as an indicator of carcinogenesis. In the present study, pulmonary adenomas occurred in all of the mice 16 weeks after the treatments with cis-DDP or L-PAM as reported earlier (11, 12). As shown in Table 1, the average number of tumor nodules induced by each anticancer agent was significantly reduced and the ratio of mice bearing tumors caused by cis-DDP was decreased by pretreatment with either MT inducer. MT concentration in the lung, which is the target tissue of carcinogenesis of cis-DDP and L-PAM, was significantly increased by zinc and bismuth and maintained at a relatively high level during treatment with the anticancer agents (Fig. 1). Elevation of the MT level in the lung by administration of the metal compounds was not affected by treatment with cis-DDP or L-PAM (data not shown). These results clearly demonstrated that pretreatment with zinc or bismuth prevented the generation of lung tumor by the anticancer agents.

It is known that MT detoxifies some heavy metals (7) and alkylating agents (13) and acts as a free radical scavenger (14). We have suggested that preinduction of renal MT by bismuth nitrate reduced renal toxicity of cis-DDP, and a distinctive correlation was found between the protective effects of bismuth nitrate on the lethal toxicity of cis-DDP and preinduced MT levels in the kidneys of mice given bismuth nitrate (2). The bone marrow toxicity of alkylating agents (5) and γ-irradiation (6) was also prevented by elevation of the MT level in bone marrow. In the present study, the injection of metal compounds induced MT and maintained it at a substantially high level in the lung during treatment with anticancer agents (Fig. 1). Thus, it is meaningful to elevate the MT level in the target tissues of carcinogenicity to protect against carcinogenesis induced by anticancer agents.

It has been reported that most alkylating agents are carcinogenic in experimental animals (1, 11). Increased risk of acute leukemia has also been suggested for patients with multiple myeloma, Hodgkin's disease, or ovarian cancer undergoing chemotherapy using alkylating agents (15-17). cis-DDP is also known as a carcinogen in experimental animals (12). In the future, secondary carcinogenesis by anticancer agents may increase because cancer chemotherapy using drugs with high carcinogenicity is widely performed in increasing numbers of patients all over the world. On the other hand, several investigators have shown that pretreatment with bismuth compounds resulted in a substantial reduction in acute toxic side effects of γ-irradiation (6), tumor necrosis factor (18), and anticancer agents such as cis-DDP (2), Adriamycin (3), cyclophosphamide (5), and bleomycin (5) without compromising their antitumor activities. Kagimoto et al. have recently reported that preadministration of bismuth nitrate prevented radiogenic thymoma induction in mice (19). Bismuth compounds induce MT synthesis specifically in normal tissues such as kidney, heart, and bone marrow but not in tumors (2, 3, 5). However, we have shown...
previously that pretreatment with zinc diminished both the antitumor activity and acute side effects of cis-DDP in tumor-bearing mice because zinc induces MT synthesis not only in the normal tissues but also in tumor tissue (5, 20). Therefore, MT induction by bismuth compounds in appropriate tissues should be a useful adjunct in cancer chemo- and radiotherapies by preventing not only dose-limiting toxicity but also secondary carcinogenic effects of anticancer agents and γ-irradiation.

It is of interest that incidence of the spontaneous tumor observed in A/J mice was also reduced significantly by zinc treatment in the present study (Table 1). This result may suggest a potency of MT induced by zinc to prevent the spontaneous tumorigenesis in A/J mice.

**Table 1 Effect of pretreatment with metal compounds on the induction of lung tumor in female A/J mice by anticancer drugs**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Pre-treatment</th>
<th>No. of mice</th>
<th>% of mice with lung tumors</th>
<th>Avg. no. of tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>33</td>
<td>0.42 ± 0.67a</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td>10</td>
<td>0b</td>
<td>0b</td>
</tr>
<tr>
<td>Bi</td>
<td></td>
<td>10</td>
<td>20</td>
<td>0.20 ± 0.42</td>
</tr>
<tr>
<td>cis-DDP</td>
<td>13</td>
<td>100</td>
<td>3.17 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>cis-DDP</td>
<td>Zn</td>
<td>13</td>
<td>69b</td>
<td>1.38 ± 1.40c</td>
</tr>
<tr>
<td>cis-DDP</td>
<td>Bi</td>
<td>13</td>
<td>54c</td>
<td>0.69 ± 0.75f</td>
</tr>
<tr>
<td>L-PAM</td>
<td>13</td>
<td>100</td>
<td>12.15 ± 1.68</td>
<td></td>
</tr>
<tr>
<td>L-PAM</td>
<td>Zn</td>
<td>12</td>
<td>100</td>
<td>5.00 ± 2.80f</td>
</tr>
<tr>
<td>L-PAM</td>
<td>Bi</td>
<td>12</td>
<td>100</td>
<td>8.83 ± 2.98f</td>
</tr>
</tbody>
</table>

* Mean ± SD.
b, c Significantly different from corresponding group without metal pretreatment (\(x^2\) test).
\(b P < 0.05.\)
\(c P < 0.01.\)

![Fig. 1. Concentration of MT in the lung of mice treated with metal compounds. The values are mean ± SD (bars) for four mice. *Significantly different from the control (\(P < 0.001, t\) test).](image)

References

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