Modulation of Resistance to Anticancer Drugs by Inhibition of Metallothionein Synthesis

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Abstract

The expression of metallothionein (MT) in certain tumor cells has been associated with resistance to anticancer drugs. In the present study, we examined the effects of inhibition of MT synthesis on resistance to anticancer drugs of human bladder tumor which were inoculated in nude mice. The results show that pretreatment of tumor-bearing mice with zinc salts increased MT content, both in normal and tumor tissues, with a marked reduction in the antitumor activity of cisplatin, Adriamyacin, and melphalan. Injection of propargylglycine, an inhibitor of cystathionase, decreased MT induction by zinc in the tumor and diminished the resistance to these drugs. These results suggest a role for MT in drug resistance in tumors, and injection of propargylglycine may provide a potential means to overcome drug resistance caused by elevation of MT levels in certain tumors.

Introduction

The two major obstacles on the therapeutic use of antineoplastic drugs have been the adverse side effects of drugs and the acquired or inherent drug resistance of the tumors. Although induction of MT synthesis has been shown to protect organs from the toxic side effects of several anticancer drugs (1), tumors with elevated MT levels showed resistance to these drugs (2). MT is a cysteine-rich low molecular weight protein with a high affinity for certain metals and is present in small basal levels in most tissues (3). The synthesis of MT can be induced by a variety of agents, including metals and hormones (3). Although the physiological role of MT is still unclear, studies have shown that MT is involved in detoxification of certain heavy metals and free radical toxicity and also in the homeostasis of essential metals such as zinc and copper (3).

Previous studies from our laboratory have shown that preinduction of MT synthesis in the target organs of toxicity by administration of bismuth compounds protected against the toxic side effects of several anticancer drugs such as cis-DDP (4—7), ADR (7, 8), bleomycin (1, 7), cyclophosphamide (1, 7), tumor necrosis factor (9), and γ-irradiation (10) without compromising their antitumor activities in mice. Cell lines expressing high MT levels are shown to be resistant to various anticancer drugs such as cis-DDP, l-PAM, chlorambucil, ADR, and ionizing radiation (2). We have recently shown that PPG (11), a specific inhibitor of cystathionase, can inhibit MT synthesis in a mouse bladder tumor model and decrease cis-DDP resistance acquired by an increase in the MT level of the tumor (12). These results suggested a role for MT in drug resistance of certain tumors.

There are other reports showing the resistance of tumors with high MT content to ADR, which is known to attack tumor cells by generating free radicals, as well as resistance to other types of anticancer drugs such as alkylating agents (1). These studies suggested that MT in the tumors can bind with alkylating agents (13) and also act as a scavenger of free radicals (14, 15). Thus, inhibition of MT synthesis in tumors may reduce the resistance to alkylating agents and free radical-generating anticancer drugs, similar to the effects observed with cis-DDP. However, it is unclear whether PPG can inhibit the induction of MT synthesis or alter cis-DDP resistance in tumors other than mouse bladder tumors. Since there are several mechanisms involved in tumor drug resistance and the presence of high MT may be one of these mechanisms, it is important to study modulation of MT synthesis in various tumor cells, especially human tumors and its effect on drug resistance. In the present study, we have examined the effect of PPG on induced synthesis of MT by injection of zinc salts in mice bearing either mouse or human tumor cells. We have also investigated the effect of alteration in MT synthesis on antitumor activities of several anticancer drugs in nude mice inoculated with human bladder tumor.

Materials and Methods

Animals and Chemicals. Five-week-old female ICR nude mice [Crl:CD-1(ICR)-nu] were supplied by Charles River Japan, Inc. (Atsugi, Japan). Seven-week-old male BALB/c and CD2F1 mice were purchased from Japan SLC (Hamamatsu, Japan). Mice (four each) were housed in a cage under specific pathogen-free conditions and were given free access to food and tap water. cis-DDP was supplied by Nippon Kayaku Co., Ltd. (Tokyo, Japan). ADR, l-PAM, and PPG were purchased from Sigma Chemical Co. (St. Louis, MO). Metal compounds and other chemicals were purchased Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Tumors. Human bladder tumor cells (NMB-1) were a gift from Dr. M. Akimoto, Department of Urology, Nippon Medical School (Tokyo, Japan) and were maintained by s.c. transplantation in the backs of female ICR nude mice. Meth-A fibrosarcoma cells were a gift from Dr. Y. Kumazawa, School of Sciences, Kitasato University (Sagamihara, Japan) and passaged by i.p. transplantation in male BALB/c mice. Colon adenocarcinoma 26 cells (colon 26) were supplied by Dr. T. Tsuruo, Institute of Molecular and Cellular Biosciences, University of Tokyo (Tokyo, Japan) and were maintained by s.c. transplantation in the backs of male BALB/c mice. The viability of tumor cells was tested by trypan blue exclusion.

Treatments and Analysis. Meth-A (2 × 10⁶ cells/mouse), colon 26 (2 × 10⁶ cells/mouse), or NMB-1 (1 × 10⁷ cells/mouse) were inoculated s.c. into the backs of CD2F1 mice (8 weeks of age) or ICR nude mice (6 weeks of age) on day 0, respectively. Seven days after tumor cell inoculation, tumor-bearing mice were randomized into control and experimental groups with four mice/group. 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 at a dose of 500 μmol/kg s.c. into the left flank region of the abdomen once a day for 3 days from days 7 to 9 (before and along with ZnSO₄ injection). MT contents in the tumors were determined using the mercury-binding assay (16) as modified by Naganuma et al. (4) and expressed as nmol mercury bound to MT on day 10 (at the time of injection of the anticancer drugs). A group of ZnSO₄- and PPG-injected mice were given an injection i.p. with one dose of...
cis-DDP (40 μmol/kg), ADR (20 μmol/kg), or L-PAM (60 μmol/kg) on day 10, respectively. The antitumor activity was evaluated by tumor weight on day 15 (5 days after the injection of anticancer drugs). The data were analyzed by Student's t test.

Results and Discussion

The concentrations of MT in two mouse tumors, Meth-A and colon 26 grown in CD2F1 and a human tumor NMB-1 grown in ICR nude mice are shown in Fig. 1. There were differences in the basal levels of MT, and both mouse tumors contained higher MT levels than the human tumor. Injection of the mice with zinc sulfate significantly increased MT levels in all of the tumors (P < 0.005). When tumor-bearing mice were injected with PPG, both the basal level and zinc-induced MT levels in Meth-A and NMB-1 tumors were reduced significantly (P < 0.005), with the effect being most prominent in the Meth-A tumor. However, the MT levels were unchanged in colon 26 tumor after injection of PPG.

The conversion of methionine to cysteine through the transsulfuration pathway is a major source of cysteine in many tissues, especially in liver. PPG has been shown to be an irreversible inhibitor of γ-cystathionase in mice, and its injection can decrease the intracellular cysteine levels (11). In rat hepatocytes, it has been reported that methionine was used as a major sulfhydryl source for the synthesis of MT via the transsulfuration pathway (17). Gallant and Cherian (18) have demonstrated that inhibition of cystathionine in newborn rats by injection with PPG decreased hepatic MT levels. They have reported previously that, in tumor-bearing mouse, injection of PPG decreased the induction of MT synthesis in both the mouse bladder tumor (MBT-2) and liver but not in the kidney (12). These studies suggested that, similar to the liver, cystathionase is present in certain tumor cells, and this may be a source of cysteine for MT synthesis. However, our present study shows that, in mice bearing colon 26 tumor cells, preadministration of PPG did not inhibit either basal MT- or zinc-induced MT synthesis, suggesting that all the tumors may not necessarily have this pathway. The specific inhibition of MT synthesis by PPG was recognized, not only in the mouse tumors such as MBT-2 and Meth-A, but also in the human bladder tumor (NMB-1). These results suggest that the cystathionase pathway may be a major source of cysteine for MT synthesis in certain tumors such as MBT-2, Meth-A, and NMB-1. In colon 26 tumors, it is possible that the dose of PPG used in our study may not be enough to reduce the intracellular cysteine level to inhibit the induced synthesis of MT, or this pathway is not present in this tumor, similar to kidney cells. The induced synthesis of MT in certain tumors and the inhibitory effect of PPG on MT synthesis should be studied, not only in various transplantable tumor cells of animal and human origin, but also in tumor cells resulting from experimental carcinogenesis to understand the role of MT in tumor biology, especially in anticancer drug resistance.

The effects of modulation of MT synthesis by ZnSO₄ and/or PPG on antitumor activities of cis-DDP, ADR, and L-PAM on the NMB-1 tumor, transplanted in ICR nude mice are shown in Fig. 2. All of these drugs were effective to inhibit the growth of this tumor. Although the tumor weights of mice treated with cis-DDP, ADR, or L-PAM were decreased to about 25% of untreated mice, pretreatment of the mice with ZnSO₄ significantly decreased the antitumor activities of these drugs (P < 0.005). However, the inhibitory effects of zinc-induced MT on the antitumor activities of these drugs were overcome by injection of PPG. In the case of L-PAM, particularly, tumor weight of mice pretreated with PPG and ZnSO₄ was the same as the tumor weight of mice injected with the drug alone. The antitumor activities of these drugs were unchanged by injection of PPG alone.

Several studies have shown that certain tumor cells with increased MT content were resistant to cis-DDP both in vitro (19–21) and in vivo (1, 12, 22). In addition, tumor cells with acquired resistance to cis-DDP frequently had an increase in MT content and overexpressed MT mRNA (21, 23), and reversal of the cis-DDP resistance phenotype is accompanied by a decrease in MT content (23). These studies suggest that MT levels in tumors may be one of the factors closely related to cis-DDP resistance in tumors. Furthermore, several investigators have reported that an increase in cellular MT synthesis resulted in resistance to several alkylating anticancer drugs (13, 21). Webber et al. (24) have reported that MT-rich and cadmium-resistant human prostate carcinoma cells showed resistance to ADR. In an in
vivo study, the increase of MT level in mouse transplantable tumors has been shown to be resistant to both alkylating agents and ADR (1). In the present study, the antitumor activities of cis-DDP, ADR, and L-PAM were also reduced in the human bladder tumor (NMB-1) transplanted s.c. into ICR nude mice, when MT content in the tumor was increased by injection of zinc. Thus, MT may be one of the important cellular factors in resistance to various anticancer agents; the presence of high levels of MT in certain tumors may be a major obstacle for effective therapy of cancer, and screening of tumors for MT is important to determine their drug resistance.

In previous studies, we have demonstrated that the inhibition of MT synthesis by PPG injection decreased the cis-DDP resistance of mouse bladder tumor, caused by an elevated level of MT. The present study confirms that, in human bladder tumor, PPG injection decreased MT induction, resulting in the reduction of cis-DDP resistance, similar to that in mouse bladder tumor cells. Moreover, we have shown that PPG injection could markedly overcome resistance to not only cis-DDP but also ADR and L-PAM acquired by an increase in MT levels in human bladder tumor cells. Our results suggest, therefore, that the combination of PPG and anticancer drugs may provide a promising protocol to enhance their efficacy in cancer chemotherapy by specifically reducing MT levels in certain tumors.

References


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