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Prevention of Carboplatin-induced Resistance in Human Ovarian Tumor Xenografts by Selenite

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Abstract. *Background/Aim: We have been exploring a prevention approach to the problem of drug resistance which develops during ovarian cancer chemotherapy. We have previously described an in vivo model of the development of resistance to the chemotherapy drug cisplatin in xenografts, and the prevention of this resistance by selenium compounds. However, a different platinum-based drug, carboplatin, is frequently utilized in ovarian cancer treatment. The aim of the present study was to design a model for the induction of resistance by carboplatin in vivo. Materials and Methods: Tumors were initiated in immunodeficient mice by subcutaneous inoculation of A2780 human ovarian tumor cells. The sensitivity of the resulting tumors to therapy was determined by measuring the effect on tumor growth of a single intraperitoneal (i.p.) treatment with a high dose of carboplatin. Results: The growth of control tumors was completely (although temporarily) stopped by this treatment; however, a single pre-treatment with a low i.p. dose of carboplatin resulted in the rapid development of resistance to carboplatin, and cross-resistance to cisplatin. Pre-treatment with selenite in addition to carboplatin prevented the induction of resistance. When cells from these pre-treated tumors were transplanted to new animals, the derivative tumors retained the sensitive or resistant phenotype of their tumor of origin. Conclusion: Selenite can prevent the induction of resistance by carboplatin in human ovarian tumors, and thus may offer an approach to extending the long-term efficacy of platinum chemotherapy.*

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Many forms of cancer are successfully treated with chemotherapy. However in some types of cancer, such as ovarian, this treatment becomes ineffective due to the development of resistance by the tumor cells to chemotherapy drugs (1, 2). The cure rate for ovarian cancer remains approximately 20%; in most patients the tumors recur with resistance to a variety of chemotherapy drugs (3), including platinum-based drugs which are the staples of ovarian cancer chemotherapy (3). Many researchers have studied mechanisms involved in tumor cell drug resistance. These include mismatch repair of Pt-DNA damage, decreased uptake or increased efflux of drug, and intracellular de-toxification, for example by glutathione (4). Several attempts have been made to overcome resistance once it has developed (5). Unfortunately, reversal treatments also tend to weaken the defenses of the patient and fail in clinical trials due to their severe toxicity (1, 2). In contrast, an agent capable of preventing tumor cells from developing resistance might not be toxic to normal cells and thus should be well-tolerated by patients (5). However, investigation of this strategy poses special challenges. In order to study the prevention of resistance, it is necessary for resistance to be induced within a narrow, predictable period of time. We have developed a model of ovarian cancer in which resistance to the platinum drug cisplatin occurs within seven days (6) which has allowed us to test potential resistance-preventive agents. However a different platinum based drug, carboplatin, is frequently utilized in ovarian cancer treatment. Carboplatin differs from cisplatin in chemical structure, activity and effective dose (7). Thus, the conditions required for the induction of resistance by carboplatin, and its prevention, should also be distinct from those of cisplatin. In this study we describe the design of a model for the induction of resistance by carboplatin and present evidence for the retention of this induced resistance, even after tumors are transplanted into a second generation of mice.

Selenium is an essential trace element which has been extensively studied for its anticancer activity (8). Although most cancer research on selenium has focused on prevention of tumor initiation and promotion (8) there have also been a

number of studies which have demonstrated the ability of inorganic and organic selenium compounds to enhance the efficacy of standard chemotherapeutic drugs (9-17). Selenium compounds have also been found to reduce drug toxicity and have been used to ameliorate the side-effects of chemotherapy (13, 18-21). In our previous studies, we described an additional effect of selenite, namely the prevention of drug resistance induced in human ovarian tumors by melphalan (22) or cisplatin (6). Here, we describe our utilization of the carboplatin model to demonstrate the ability of selenite to prevent carboplatin-induced resistance.

Materials and Methods

Chemicals. Cisplatin, carboplatin and sodium selenite were purchased from Sigma/Aldrich (St. Louis, MO, USA).

Cells. The human ovarian tumor cell line A2780 was obtained from Dr. Thomas Hamilton, Fox Chase Cancer Center, Philadelphia, PA, USA. Cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12, purchased from GIBCO Life Sciences (Grand Island, NY, USA). Cultures were maintained in 10% or 15% fetal bovine serum (Atlanta Biologicals, Norcross, GA, USA) and Penicillin/Streptomycin (GIBCO Life Sciences). Cell cultures were maintained at 37˚C with 5% CO_2.

Drug sensitivity of tumor xenografts. Female athymic nude mice (Harlan Sprague-Dawley, Indianapolis, IN, USA) were inoculated subcutaneously (*s.c.*) in the flank with 0.1 ml of a cell suspension containing 1×10^6 A2780 cells. Tumor dimensions were measured with calipers and the volume was calculated using the formula: Volume=length × width^2/2. After exponential growth of the tumors had been established, the tumor-bearing mice were inoculated *i.p.* with either carboplatin (50 mg/kg) or cisplatin (7.2 mg/kg) and measurement of tumor size was continued for 3 days. Tumors in which growth ceased after inoculation with the drug were considered sensitive to the drug, those in which growth continued were considered resistant.

Tumor transplantation. Tumor-bearing mice were sacrificed, tumors were removed and fragments were homogenized in culture medium containing Penicillin/Streptomycin and 15% fetal bovine serum. The cell suspension was seeded into culture flasks and incubated for two weeks (without passage). Cells were removed from the flasks with trypsin, and 0.1 ml of a cell suspension containing 5×10^6 cells was inoculated *s.c.* into fresh mice. After exponential growth of the transplant tumors had been established they were tested for drug sensitivity as described above.

All experiments involving animals were approved by the Rutgers University Animal Welfare Committee and were carried out under the supervision of the university veterinarians.

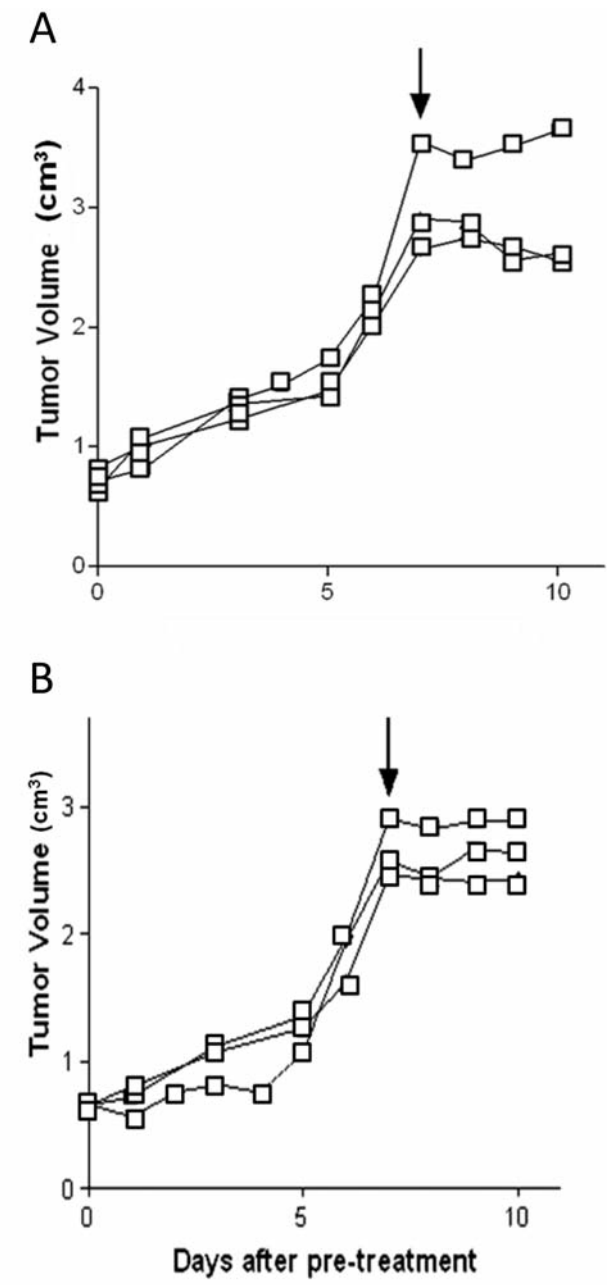

Figure 1. *Response of control tumor xenografts to platinum compounds. Xenografts were initiated from A2780 cells as described in the Materials and Methods. Tumor-bearing animals received a single i.p. treatment with PBS (day 0) followed on day 7 (arrow) by carboplatin (50 mg/kg) (A) or cisplatin (7.2 mg/kg) (B). Each curve shows the tumor response of an individual animal.*

Results

Induction of resistance. In order to study the development of resistance, it was first necessary to determine the doses of chemotherapy drugs that are effective against control A2780 xenografts. For this purpose, xenografts were initiated with A2780 cells, as described in the Materials and Methods. Tumor-bearing mice received a single *i.p.* pre-treatment of PBS (day 0) followed by either carboplatin (50 mg/kg) or cisplatin (7.2 mg/kg) on day 7. Tumor volumes were measured every 24-48 h following pre-treatment and tumor growth curves of

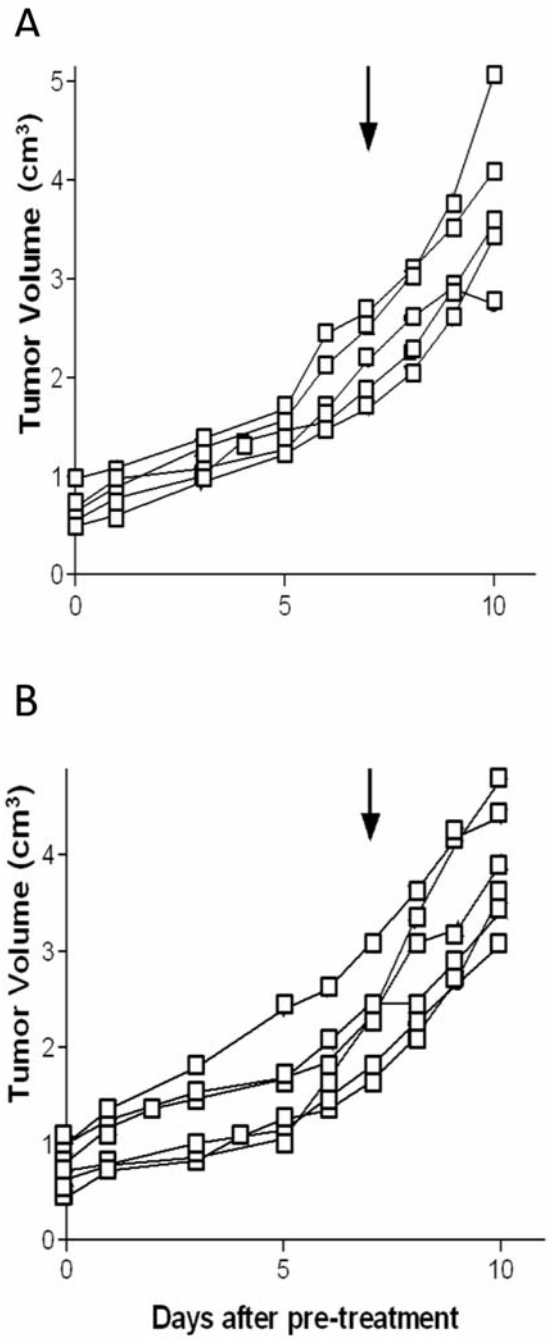

Figure 2. *Response of carboplatin pre-treated tumors to platinum compounds. Xenografts were initiated from A2780 cells as described in the Materials and Methods. Tumor-bearing animals received a single i.p. treatment with carboplatin (15 mg/kg) (day 0) followed on day 7 (arrow) by carboplatin (50 mg/kg) (A), or cisplatin (7.2 mg/kg) (B). Each curve shows the tumor response of an individual animal.*

individual animals were obtained. The results (Figure 1) indicate that these doses of carboplatin and cisplatin were effective in inhibiting tumor growth for at least three days.

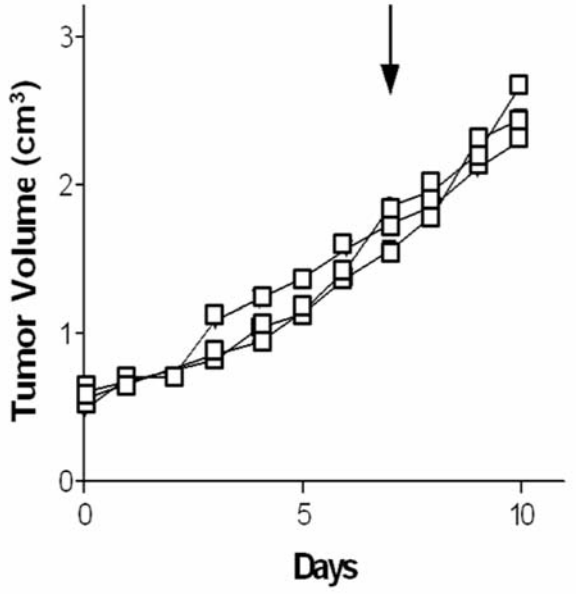

Figure 3. *The resistant phenotype is maintained in transplanted tumors. Xenografts were initiated from A2780 cells as described in the Materials and Methods. Tumor-bearing mice received a single i.p. treatment with carboplatin (15 mg/kg) as in the experiment shown in Figure 2. The animals were sacrificed seven days later (without exposure to the high dose of the drug). The tumors were excised and transplanted into new mice as described in the Materials and Methods. After tumor growth was clearly established, the animals received a single i.p. treatment with carboplatin (50 mg/kg) (arrow). Each curve shows the tumor response of an individual animal.*

To determine whether pre-treatment with a low dose of carboplatin induces resistance to these high doses, mice were pre-treated on day 0 with 15 mg/kg carboplatin, followed by a high dose of carboplatin (50 mg/kg) or cisplatin (7.2 mg/kg) on day 7. The results shown in Figure 2 demonstrate that a single low dose of carboplatin induced resistance to a subsequent treatment with a high dose of carboplatin (Figure 2A) and also induced cross-resistance to a high-dose of cisplatin (Figure 2B).

Maintenance of resistance following transplantation. To determine whether this induced resistance is short-lived or is maintained by the tumor after transplantation, tumor-bearing mice were sacrificed seven days after the resistance-inducing low dose of carboplatin (without exposure to a high dose of the drug). The tumors were homogenized, and the cells were then prepared and injected into mice as described in Materials and Methods. Following tumor appearance, tumor growth was measured and the tumors were tested for sensitivity to high dose carboplatin. The results show that transplants of tumors which had been pre-treated with carboplatin were also resistant to carboplatin (Figure 3); the carboplatin-resistant phenotype was maintained.

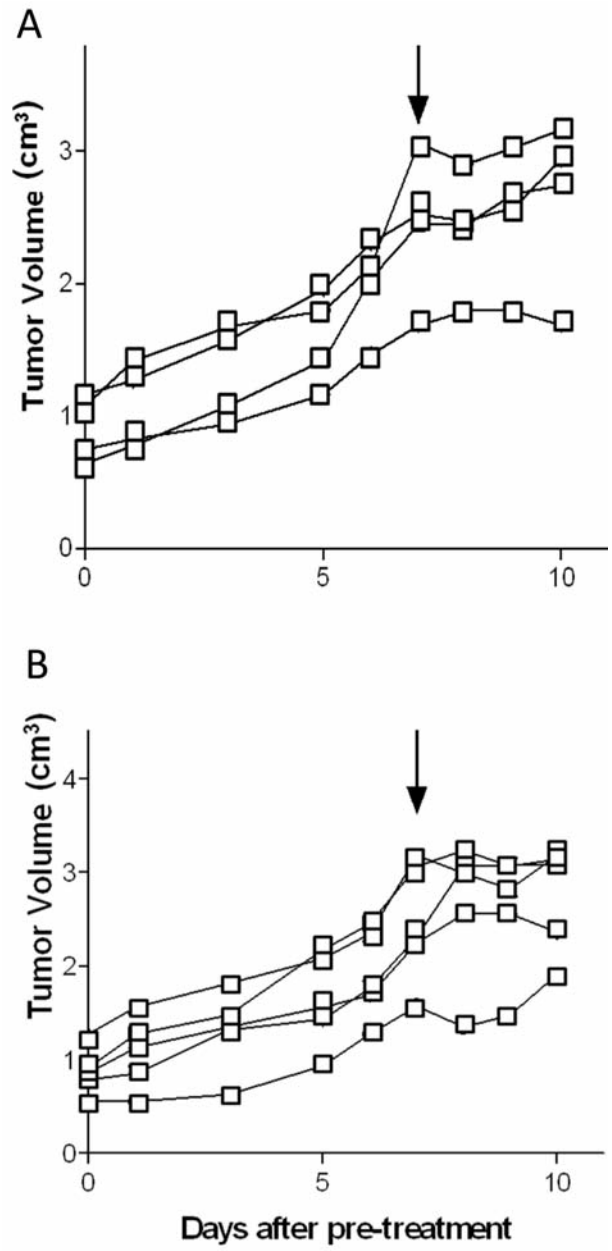

Figure 4. *Selenite prevents the induction of resistance by carboplatin. Xenografts were initiated from A2780 cells as described in the Materials and Methods. Tumor-bearing animals received a single i.p. treatment with carboplatin (15 mg/kg) (day 0) and 3 i.p. treatments with selenite (1.5 mg/kg) on days -1, 0 and +1, followed on day 7 (arrow) by carboplatin (50 mg/kg) (A) or cisplatin (7.2 mg/kg) (B). Each curve shows the tumor response of an individual animal.*

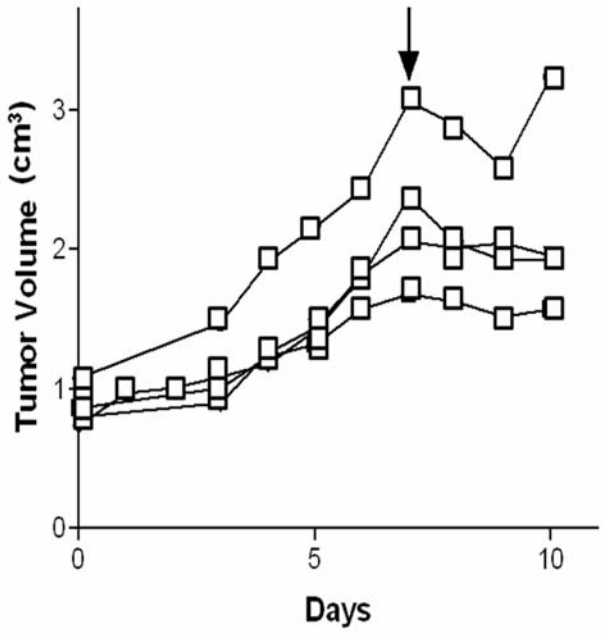

Figure 5. *The sensitive phenotype is maintained in transplanted tumors. Xenografts were initiated from A2780 cells as described in the Materials and Methods. Tumor-bearing mice received a single i.p. treatment with carboplatin (15 mg/kg) and 3 i.p. treatments with selenite (1.5 mg/kg), as in the experiment shown in Figure 4. The animals were sacrificed seven days later (without exposure to the high dose of the drug). The tumors were excised and transplanted into new mice as described in Materials and Methods. After tumor growth was clearly established, the animals received a single i.p. treatment with carboplatin (50 mg/kg) (arrow). Each curve shows the tumor response of an individual animal.*

Prevention of induced resistance. In order to test whether the resistance induced by low-dose carboplatin could be prevented by administering selenite along with the carboplatin pre-treatment, mice bearing A2780 tumor xenografts were pretreated on day 0 with carboplatin, and were also given three *i.p.* treatments with selenite. Each selenite injection contained 1.5 mg/kg, and was administered on days -1, 0 and +1. On day 7, all mice received *i.p.* high-dose carboplatin or cisplatin. The results show that the inclusion of selenite in the pre-treatment prevented the induction of resistance to carboplatin (Figure 4A), as well as cross-resistance to cisplatin (Figure 4B).

Maintenance of sensitivity following transplantation. To determine whether tumors in which resistance had been prevented would retain their sensitivity to chemotherapy after being transplanted into a second group of mice, tumors were removed from mice sacrificed on day 7 after the combination pre-treatment with selenite and carboplatin. These tumors were then transplanted into new mice, as described in Materials and Methods. Following tumor appearance, tumor growth was measured and tumors tested for sensitivity to high-dose carboplatin. The results shown in Figure 5 indicate that the transplanted tumors retained their sensitivity to carboplatin.

Discussion

Platinum compounds are part of standard chemotherapy care for advanced ovarian cancer, and carboplatin is the preferred compound at many clinical institutions. However, development of resistance remains a serious obstacle to achieving long-term cure. Our studies with human ovarian tumor xenografts have demonstrated the induction by carboplatin of resistance to subsequent treatment with either carboplatin or cisplatin. These studies have also demonstrated that the induction of resistance can be prevented by inclusion of selenite in the initial treatment. Thus the prevention of resistance by selenite may offer an approach to extending the long-term efficacy of platinum chemotherapy (23). Our studies further suggest that the inclusion of selenite during initial chemotherapy can prevent resistance even in a recurrent tumor. It should be noted that selenium compounds have been shown to have a protective effect against platinum toxicity while not interfering with its chemotherapeutic efficacy (13, 18-21), thus mitigating concerns about potential toxicity. Thus, the combination of carboplatin and selenite is appropriate for clinical testing; the potential benefits of inclusion of selenite as part of a platinum-based chemotherapy protocol for initial treatment of ovarian cancer has been investigated in a Phase 1 clinical trial (24).

There have been many studies on the mechanisms of resistance to platinum compounds (2-4). These studies have provided evidence that, at least in some cases, resistance is the result of elevated levels of cellular glutathione, which may result from an increase in the expression of the gene for γ-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in glutathione biosynthesis (25-27). In our previous studies, we obtained evidence that induction of resistance in ovarian xenografts is accompanied by an increase in glutathione, and that buthionine sulfoximine, an inhibitor of γ-GCS can eliminate this resistance (6). Thus, we have hypothesized that the prevention of platinum resistance may result from an effect of selenite on the expression of this enzyme. Preliminary results of a microarray analysis have shown that the level of expression of γ-GCS in cells derived from carboplatin-pretreated tumors is more than twice that in untreated control cells. In contrast, the level of expression in cells derived from tumors pre-treated with both carboplatin and selenite is identical to that of untreated control cells. The fact that transplants of the resistant and sensitive tumors maintain their respective tumor phenotypes during transplantation (Figure 3) further suggests that prevention of resistance may result from a genetic or epigenetic effect of selenite. These possibilities are currently under investigation.

Acknowledgements

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