

Selenite Enhances and Prolongs the Efficacy of Cisplatin Treatment of Human Ovarian Tumor Xenografts

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Abstract. *Background/Aim: Our earlier studies on ovarian tumor xenografts provide evidence that co-treatment with selenite prevents the development of resistance to single-treatment using the drug cisplatin. However, these studies did not reflect the repetitive schedule of clinical chemotherapy. We hypothesized that selenite can enhance the effectiveness of cisplatin during the course of repeated treatments, reflecting clinical practices. Materials and Methods: Multiple i.p. injections of cisplatin (5.2 mg/kg) alone, or with selenite (1.5 mg/kg), were administered to mice bearing subcutaneous xenografts of human ovarian tumor (A2780) cells and the tumor volume was recorded. Results: Selenite increased and prolonged the efficacy of multiple cisplatin treatments, although selenite was not an effective inhibitor by itself. In the absence of selenite, the effectiveness of cisplatin decreased. Conclusion: The ability of selenite to prolong the effectiveness of repetitive cisplatin treatment, most likely by preventing the development of resistance, makes it a strong candidate for inclusion in clinical trials.*

Early-stage ovarian cancer is often asymptomatic. As a result, patients frequently have advanced disease at the time of diagnosis and require chemotherapy (1). However, for most patients there is no effect of chemotherapy and they experience a recurrence within a relatively short period of time (2). As a result, mortality is approximately 65% of the incidence rate and ovarian cancer is one of the leading causes of cancer death in women (3). Thus, there is an urgent need to improve the efficacy of chemotherapy for ovarian cancer.

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Since a platinum compound is a component of virtually all standard protocols for chemotherapy of ovarian cancer (3), an enhancement of the effectiveness of such compounds would be of great clinical significance.

Our studies investigating the potential uses of selenium compounds in the treatment of ovarian cancer (4-8) have led us to hypothesize that selenite may be able to increase the efficacy of cisplatin. However, previous methods used in our studies did not reflect the repetitive schedule of chemotherapy treatments. Thus, there was a need to determine whether selenite could improve the efficacy of cisplatin during repeated treatments. In this article we describe studies which demonstrate that co-treatment of selenite enhances the efficacy of cisplatin treatment in human ovarian tumor xenografts, and that prolonged treatment with this combination remains more effective than the same treatment schedule with cisplatin alone.

Materials and Methods

A2780 human ovarian tumor cells were grown in culture as described previously (8). Female athymic nude mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA) and were housed in sterile microisolator cages. At 5-6 weeks of age, the animals were inoculated once subcutaneously (s.c.) in the flank with 0.1 ml of a cell suspension containing 5×10^6 A2780 tumor cells. Tumor dimensions were measured with calipers and the volume was calculated using the formula: Volume = length \times width²/2.

The treatment protocol was begun after the tumors had reached a size of approximately 0.5 cm³. Animals were injected intraperitoneally (*i.p.*) twice weekly (days 1 and 4 of each week) either with Phosphate Buffered Saline (PBS) or with 5.2 mg/kg cisplatin (Sigma, St. Louis, MO, USA). Those animals which received selenite (Sigma, St. Louis, MO, USA), either alone or in combination with cisplatin were injected with 1.5 mg/kg *i.p.* thrice weekly. (The animals treated with selenite alone were injected on days 1, 2 and 3 of each week; those treated with selenite in combination with cisplatin were injected with selenite one day before, 4 hours before and 1 day after the first cisplatin treatment of the week).

The results are presented as the mean \pm SD for the animals in each treatment group. The growth rates were calculated by non-linear regression (exponential growth model) using GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA).

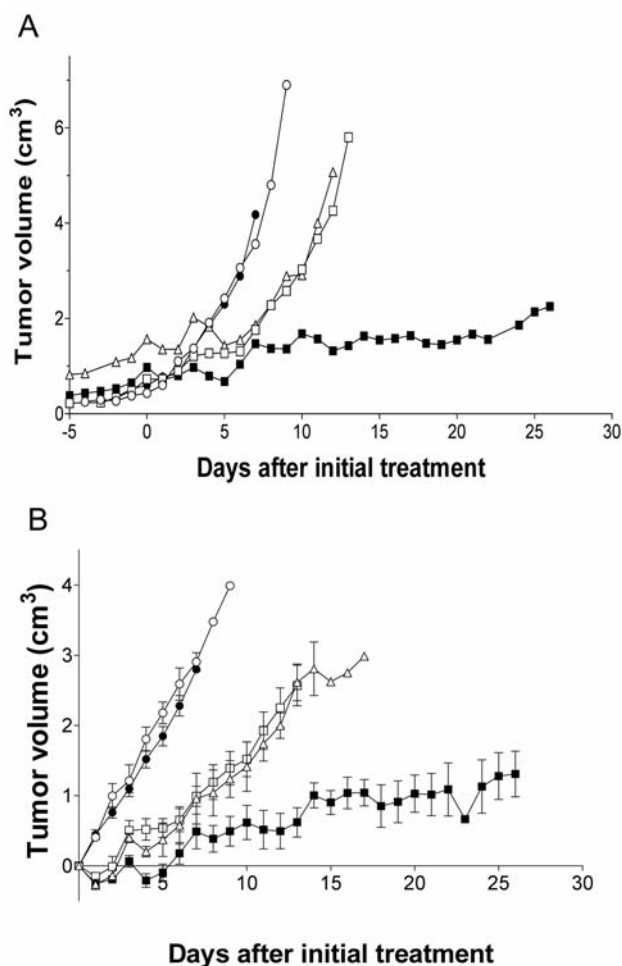


Figure 1. Enhancement of the efficacy of cisplatin by selenite. Tumor-bearing animals were treated with the indicated regimen, as described in the Materials and Methods, beginning on day 0. A: Results for representative individual animals. The arrows indicate the time of cisplatin (or PBS) injection. B: The number of volume doublings was calculated each day for each animal from the formula, $\text{Volume doublings} = 3.32 \log (V/V_0)$, where V is the tumor volume on the particular day and V_0 is the volume on day 0. The results are presented as the mean \pm SD for the animals in each treatment group (4-6 animals/group). O: PBS; □: cisplatin alone (5.2 mg/kg); ■: cisplatin (5.2 mg/kg) + selenite (1.5 mg/kg); △: cisplatin (5.2 mg/kg) + sulfite (1.5 mg/kg); ◆: selenite alone (1.5 mg/kg).

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

Results

In order to investigate the hypothesis that selenite can enhance and prolong the chemotherapeutic effectiveness of cisplatin, we examined the effect of the drug alone and in combination with selenite on the growth of human ovarian tumor

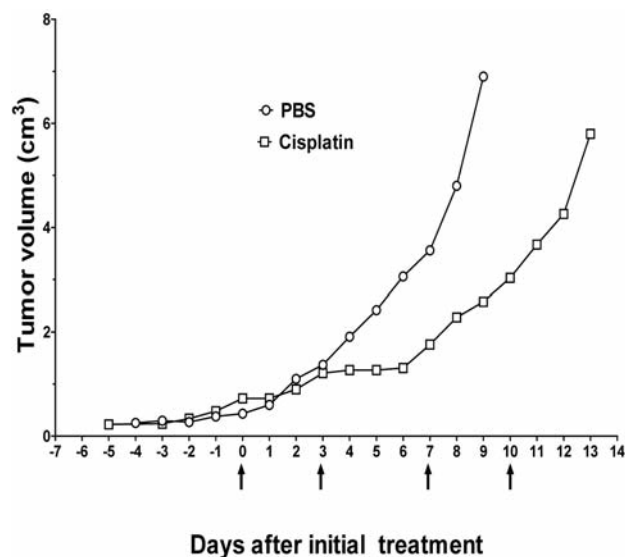


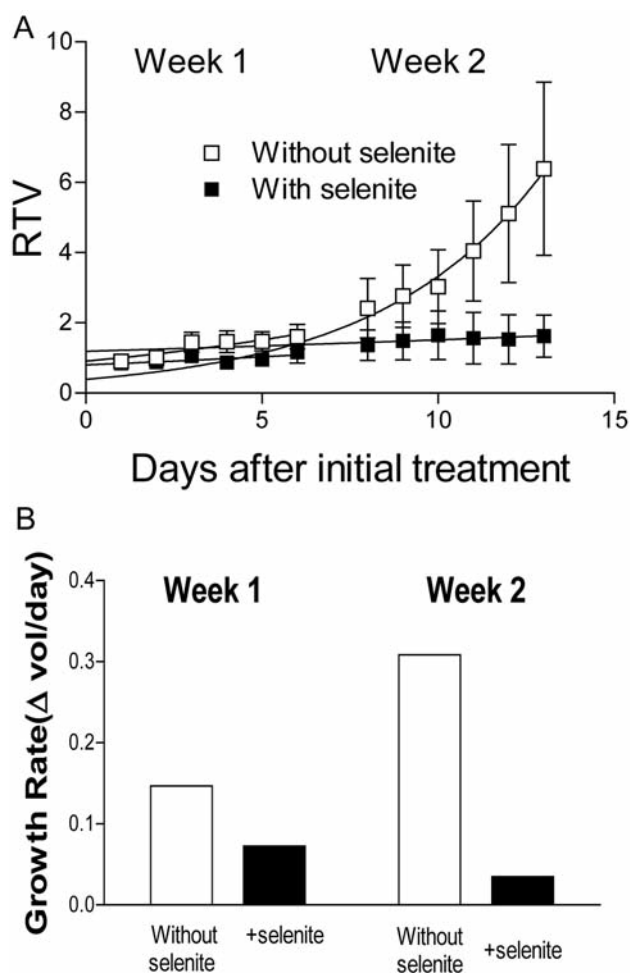
Figure 2. Decrease in the effectiveness of cisplatin during therapy. Tumor-bearing animals were injected *i.p.* on the days indicated with an arrow, with either PBS or cisplatin (5.2 mg/kg).

xenografts. The treatment protocol consisted of *i.p.* injections of 5.2 mg/kg cisplatin (twice weekly), and/or 1.5 mg/kg selenite (thrice weekly), as described in the Materials and Methods. The results (Figure 1) show that while cisplatin alone did have an effect on tumor growth (in comparison to the growth of tumors treated with PBS), inclusion of selenite in the treatment protocol resulted in a significant enhancement, even though selenite by itself, had no effect on tumor growth. This enhancement was specific for the selenium compound since inclusion of the sulfur analog of selenite in the protocol did not result in any change in the efficacy of cisplatin.

Tumor growth curves for the first two weeks of therapy with PBS or cisplatin are shown for two individual animals in Figure 2. There is a clear suggestion that there was a gradual loss of the ability of cisplatin to inhibit tumor growth during the course of treatment. Thus, the enhancement of the efficacy of cisplatin by selenite (Figure 1) could result from prolongation of the effectiveness of the drug. If this is correct, then the effect of selenite should increase as the course of treatment progresses. The relative effectiveness of cisplatin alone and in combination with selenite during the first and second weeks of therapy is shown in Figure 3. It is clear that while the effect of selenite is relatively small during the first week of chemotherapy, it is much more pronounced during the second week.

Discussion

Since resistance to cisplatin appears to be developing during the course of the present treatment protocol (see Figure 2), the ability of selenite to enhance the efficacy of cisplatin is



Cisplatin treatment

Figure 3. Efficacy of cisplatin alone (5.2 mg/kg) and combined with selenite (1.5 mg/kg) during the first and the second week of therapy. A: Relative tumor volume was calculated for each tumor by dividing its daily measured volume by its volume on day 0. The results are presented as the mean \pm SD for the animals in each treatment group. B: The growth rates were calculated by non-linear regression (exponential growth model) using GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA).

likely to be the result of its prolonging the effectiveness of the drug, by preventing the induction of resistance. Thus, our results may represent an example of the strategy which we have proposed for the circumvention of drug resistance (8).

We have previously reported that approximately one week after a single treatment with a subtherapeutic dose (2.6 mg/kg) of cisplatin, a new treatment with a single higher dose of the drug was ineffective in inhibiting tumor growth (9); that is, an initial treatment with the drug induced resistance in the tumors to a subsequent treatment. Furthermore, inclusion of selenite during the initial treatment prevented the development of

resistance (9). Our findings on human tumor xenografts, indicate that a clinical investigation of the potential of selenium compounds for enhancing the effectiveness of chemotherapy is warranted. The combination of cisplatin and selenite may be particularly appropriate, since selenium compounds have been shown to have a protective effect against platinum toxicity in both animal and clinical studies (10-16). A phase I clinical trial to examine platinum/selenium combination chemotherapy has been conducted (17) and a phase II trial is currently under development.

Our results also indicated that at least in a xenograft model, there is a very rapid induction of resistance during platinum chemotherapy (see Figure 2). Clinical drug resistance is usually considered to fall into two categories: acquired and intrinsic [see for example (18, 19)]. Acquired resistance is defined as the one stemming from exposure to a drug, that results in a change in the tumor cell population from predominantly sensitive to predominantly resistant. In contrast, intrinsic resistance is considered to be present in chemo-naïve tumors, *i.e.* those that have never been exposed to the drug. However, in ovarian cancer, the initial clinical evaluation of the effectiveness of platinum treatment is made several months after the initiation of chemotherapy; if clinical resistance can in fact develop rapidly, the clinical tumors would falsely appear to be intrinsically resistant. Thus, it may be that some cases which have been classified clinically as being intrinsically resistant to cisplatin may actually reflect rapidly acquired resistance, which may be preventable. This question will also be investigated during the planned clinical trial.

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