Selenite Cytotoxicity in Drug Resistant and Nonresistant Human Ovarian Tumor Cells

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ABSTRACT

Our previous studies on selenite cytotoxicity led us to hypothesize that drug resistant tumor cells with high intracellular glutathione will exhibit a high degree of sensitivity to selenite. To examine this we studied the effects of selenite on drug resistant human ovarian tumor (NIH:OVCAR-3) cells in three assays: cytoxicity; proliferation; cell viability (trypan blue exclusion); and attachment to a solid matrix. The cells were sensitive to low levels of selenite concentrations as low as 5 μM inhibited cell proliferation and attachment; and viability was decreased by concentrations as low as 20 μM. In each of these assays the NIH:OVCAR-3 cells were more sensitive to selenite than were drug sensitive human ovarian tumor (A2780) cells. These results suggest the potential for the utilization of selenite in the treatment of some drug resistant tumors.

INTRODUCTION

Clinical resistance to chemotherapeutic drugs is a major concern in the treatment of cancer. While treatment with a combination of drugs is frequently used to circumvent resistance to a single agent (1, 2), the rate of relapse for many forms of cancer remains high. The development of tumor cell drug resistance is considered a major cause for the failure of cancer therapy (3).

The need to prevent or reverse this resistance has led to numerous studies investigating the processes responsible for its development. It has been suggested that more than one mechanism may be involved (4). For example, the overexpression of P-glycoprotein, which results in decreased accumulation of anticancer drugs (5, 6), has been shown to be a significant mechanism of drug resistance in some tumor cells (5, 7, 8). It has also been suggested that GSH, the predominant nonprotein sulfhydryl compound, is involved in resistance (9, 10), perhaps by facilitating the metabolism of drugs to less active species in the tumor cells (11, 12).

In many cases high levels of cell GSH and its associated enzymes (such as glutathione S-transferase) have been shown to occur in drug resistant tumor cells (13, 14). For example, a line of human ovarian tumor cells (NIH:OVCAR-3) derived from a patient refractory to treatment with doxorubicin, cisplatin, and cyclophosphamide (15) has high levels of GSH compared to a line of ovarian tumor cells (A2780) derived from a tumor sensitive to such drug treatment (15, 16). In addition, sublines of these sensitive (A2780) cells which were selected in vitro for drug resistance were found to contain increased levels of GSH (16). Depletion of resistant ovarian cell lines of GSH with buthionine sulfoximine resulted in an increase in their drug sensitivity (16-19).

Selenium compounds are known to act as anticancer agents, both in intact animals and in cellular systems (20, 21). The chemopreventive action of selenium compounds has been suggested to result from inhibitory effects on carcinogen activation and the potentiation of the immune system (20). In addition, compounds such as selenite induce a number of cytotoxic effects in tumor cells in vitro (22-24), which suggests that this may be one of the mechanisms involved in the inhibition of tumor development in vivo.

A number of studies have shown that sulfhydryl compounds, such as GSH, enhance selenite cytotoxicity (25-28) and that a sufficiently high level of intracellular SH compounds is required for selenite to exert certain cytotoxic effects (29-32). Thus, with selenite, in contrast to most xenobiotics, high cellular GSH correlates with high toxicity. This has led us to hypothesize that selenite should be effective against drug resistant tumor cells with high GSH levels which are resistant to other chemotherapeutic drugs. To test this hypothesis we have studied the effects of selenite on NIH:OVCAR-3 and A2780 cells, using assays which measure cell viability (the ability to exclude trypan blue), cell proliferation, and attachment to a solid matrix.

MATERIALS AND METHODS

Chemicals and Cells. Sodium selenite was purchased from BDH Chemicals, Ltd. Doxorubicin was obtained from Sigma. The NIH:OVCAR-3 cells were obtained from the American Type Culture Collection, Rockville, MD. The A2780 cells were obtained from the Fox Chase Cancer Institute, Philadelphia, PA. Both cell lines were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 medium with 1% insulin and 20% fetal bovine serum (non-heat inactivated; GIBCO) at 37°C in a 5% CO2 atmosphere. Under these conditions the NIH:OVCAR-3 cells had a doubling time of 66 h and the A2780 cells had a doubling time of 22 h.

Viability Assay. Approximately 4 x 10⁵ NIH:OVCAR-3 cells or 2 x 10⁶ A2780 cells were seeded with culture medium in 60-mm dishes, such that there were 2 x 10⁴ attached cells 4 h after seeding. At that time, the medium was replaced by medium containing selenite or doxorubicin as indicated in the individual experiments. In the long term exposure assays, the concentrations were maintained by daily changes in medium. After incubation with selenite or doxorubicin for the indicated times, cells were trypsinized and diluted with a solution of trypan blue (final concentration, 0.4%), and the number of cells excluding the dye was determined.

Proliferation Assay. Cells were seeded as above. After incubation for either 1 day (A2780) or 4 days (NIH:OVCAR-3) the medium was replaced by medium containing selenite or doxorubicin as indicated in the individual experiments and the cells were incubated for 24 h. The cells were then trypsinized, resuspended in fresh medium containing no added selenite, and seeded into 60-mm dishes at 5 x 10⁴ cells/dish. Cells were trypsinized and counted every 24 h for 6 days (A2780) or every 48 h for 11 days (NIH:OVCAR-3). Population doublings (PD) were calculated as

\[
PD = 3.32 \left( \frac{\log \text{Final count}}{\log \text{Initial count}} \right)
\]
Attachment Assay. Cells were seeded as above. After incubation for either 2 days (A2780) or 5 days (NIH:OVCAR-3) the medium was replaced with medium containing selenite or doxorubicin as indicated in the individual experiments. After incubation for 2 h the cells were trypsinized and counted and $6 \times 10^5$ cells were seeded in 60-mm dishes with medium containing no added selenite or doxorubicin. At various times the dishes were washed to remove unattached cells, and the attached cells were trypsinized and counted.

RESULTS

We examined the effect of selenite on the viability of drug resistant NIH:OVCAR-3 cells as determined by the ability of cells to exclude trypan blue. Cells were incubated with selenite for a 6-h exposure period and their viability was determined as described in “Materials and Methods.” The results (Fig. 1) show a significant decrease in the number of viable cells following exposure to selenite.

This study demonstrates the effect of short term exposure to selenite on cell viability. Because no significant proliferation occurs in these cells during the 6-h exposure period, this assay measures effects on viability independent of any effects of selenite on cell proliferation. However, the concentrations of selenite required for this assay may be prohibitively high for in vivo applications. Long term exposure to lower concentrations of selenite may provide better insight into the applicability of selenite for in vivo treatment. Thus, we exposed NIH:OVCAR-3 cells to relatively low concentrations of selenite for 5 days. There was a dose dependent decrease in the number of viable cells, as measured by their ability to exclude trypan blue (Fig. 2). Even in cultures exposed to the lowest concentration (20 μM) there were fewer cells after 2 days than were initially seeded. After 5 days of exposure to 40 μM selenite there was less than 5% of the initial number of cells.

During this 5-day exposure period significant proliferation occurred in the control cultures (i.e., those not exposed to selenite). Thus it is not possible to determine whether selenite exclusively affected cell viability (as defined as the ability to exclude trypan blue) or whether there was also inhibition of the proliferation of the cells which remained viable.

To investigate the effects of selenite on proliferation which are independent of effects on viability, cells were exposed to selenite for 24 h, after which they were trypsinized and seeded in fresh medium with no added selenite. The number of cells/dish was determined at intervals after seeding and the number of population doublings which had occurred was determined (see “Materials and Methods”). This preexposure to selenite resulted in a dose dependent inhibition of cell proliferation (Fig. 3), with significant inhibition even with concentrations of selenite as low as 7.5 μM. Thus, selenite decreases the number of viable cells in a population and also inhibits the proliferation of those cells which remain viable.

Recent studies conducted in our laboratory have demonstrated that selenite inhibits the attachment of HeLa cells to a
cells, caused only a 30% decrease in the viability of A2780 cells. Approximately 500 μM selenite was required to cause a 70% decrease in A2780 cell viability (Fig. 5).

Exposure to lower concentrations of selenite for longer periods of time (5 days) resulted in an even greater difference in sensitivity between these two cell lines (Table 2). Following exposure to 40 μM selenite the number of viable NIH:OVCAR-3 cells decreased by 97% compared to the number of cells initially exposed, whereas the number of A2780 cells increased almost 7-fold. Selenite exposure of NIH:OVCAR-3 cells resulted in a 100-fold decrease in the ratio of final to initial cell number compared to control cells; with A2780 cells, only a 4-fold decrease occurred (Table 2).

Similar results were obtained in the proliferation and attachment assays. As shown in Fig. 6, 50% inhibition of proliferation by NIH:OVCAR-3 cells occurred with 7 μM selenite, whereas the same degree of inhibition of A2780 cells required approximately 13 μM selenite. Fig. 7 shows that 50% inhibition of NIH:OVCAR-3 cell attachment occurred at concentrations of less than 7 μM selenite, compared to approximately 12 μM for A2780 cells. Thus, in all three in vitro toxicity assays the NIH:OVCAR-3 cells were more resistant to doxorubicin but more sensitive to selenite than were A2780 cells.

**DISCUSSION**

Cellular GSH and its related enzymes are involved in providing protection against most exogenous agents (11). Thus, it is not surprising that drug resistance is often accompanied by high GSH levels (14). There are, however, certain cytotoxic agents

Variation of extracellular matrix proteins. Since it has been suggested that cell attachment is an important step in tumor cell invasion and metastasis (33), inhibition of attachment may be a significant cytotoxic effect. Therefore, we examined the effect of selenite on attachment by NIH:OVCAR-3 cells. The results showed that these cells were extremely sensitive to this effect of selenite; inhibition of attachment occurred at concentrations as low as 5 μM (Fig. 4).

We have hypothesized that the drug resistant NIH:OVCAR-3 cells will be more sensitive to selenite than will comparable drug sensitive ovarian tumor cells. To investigate this we examined A2780 cells, which were derived from a non-drug resistant ovarian tumor (15). We first examined whether these two cell types retain their relative sensitivity to doxorubicin in vitro in the three assays of cytotoxicity which we have used. We found that the NIH:OVCAR-3 cells were less sensitive to the effects of doxorubicin than were the A2780 cells in all three assays (Table 1). This relative sensitivity to doxorubicin reflects the relative sensitivities of the tumors from which the cell cultures were derived (15). This suggests that the assays used in this study may be indicative of the relative sensitivity of tumors to chemotherapeutic drugs in vivo.

The effect of 6-h exposure to selenite on the viability of these two cell lines is shown in Fig. 5. The drug resistant NIH:OVCAR-3 cells were more sensitive to selenite than were the drug sensitive A2780 cells. The concentration of 100 μM selenite, which resulted in a 70% decrease in viability of NIH:OVCAR-3

Table 1 Effect of doxorubicin on A2780 cells and NIH:OVCAR-3 cells

<table>
<thead>
<tr>
<th>Assay</th>
<th>NIH:OVCAR-3</th>
<th>A2780</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability</td>
<td>5.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Proliferation</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Attachment</td>
<td>1.0</td>
<td>0.5</td>
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</tbody>
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which require GSH for their activity. In those cases, high levels of cellular GSH would be expected to be associated with a higher degree of sensitivity to these agents. This prediction has been experimentally confirmed for two such agents: neocarzinostatin (34) and selenite (32).5 These observations led us to hypothesize that drug resistant tumor cells with high GSH will exhibit a particularly high degree of sensitivity to the cytotoxic effects of selenite. This prediction has been confirmed for the ovarian tumor cells examined in this study.

Our results indicate that in these drug resistant cells several cytotoxic effects occur at very low selenite levels. For example, cell attachment was inhibited by concentrations as low as 5 \( \mu M \). Even the concentrations required to decrease cell viability were relatively low, with long term exposure. Thus, exposure to 40 \( \mu M \) selenite for 5 days resulted in a 97% decrease in the number of viable cells compared to the initial number of viable cells in the culture. Although these selenite concentrations cannot necessarily be assumed to reflect doses which will be effective in vivo, they are nevertheless well below those selenium concentrations in human blood which are known to cause acute toxicity (35).

Because of the prevalence of drug resistance in cases of advanced cancer, there exists an urgent need to develop approaches to reverse or prevent it. This study represents the first step in the evaluation of selenite as a treatment for drug resistant tumors. We have used three assays which measure different aspects of cytotoxicity in vitro. In each of these assays the cell cultures reflect the relative sensitivities to doxorubicin of the tumors from which they were derived (Table 1). In each of these assays the drug resistant cells were more sensitive to selenite, which suggests that drug resistant tumors may prove to be sensitive to selenite in vivo. This possibility is currently under investigation.

ACKNOWLEDGMENTS

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16. Louie, K., Behrens, B., Kinnel, J., Hamilton, T., Grotzing, K., McCoy, W., Young, R., and Ozols, R. Radiation survival parameters of antineoplastic proteins and proliferation. The experiment was conducted as in Fig. 3. The rates of proliferation were calculated from the best fit lines determined by regression analysis and expressed as a percentage of the rates of the control cells. The symbols are the same as in Fig. 5.

Fig. 6. Comparison of the effect of selenite on NIH:OV/AR-3 and A2780 cell proliferation. The experiment was conducted as in Fig. 3. The rates of proliferation were calculated from the best fit lines determined by regression analysis and expressed as a percentage of the rates of the control cells. The symbols are the same as in Fig. 5.

Fig. 7. Comparison of the effect of selenite on NIH:OV/AR-3 and A2780 cell attachment. The experiment was conducted as in Fig. 4. The rates of attachment were calculated from the best fit lines determined by regression analysis and expressed as a percentage of the rates of the control cells. The symbols are the same as in Fig. 5.

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