Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine

Karen G. Louie, Brent C. Behrens, Timothy J. Kinsella, Thomas C. Hamilton, Karen R. Grotzinger, Wilma M. McKay, Margaret A. Winker, and Robert F. Ozols

ABSTRACT

The optimum integration of chemotherapy and irradiation is of potential clinical significance in the treatment of ovarian cancer. A series of human ovarian cancer cell lines have been developed in which dose-response relationships to standard anticancer drugs have been determined, and the patterns of cross-resistance between these drugs and irradiation have been established. By stepwise incubation with drugs, sublines of A2780, a drug-sensitive cell line, have been made 100-fold, 10-fold, and 10-fold more resistant to Adriamycin (2780AD), melphalan (2780ME), and cisplatin (2780CP). Two additional cell lines, NIH:OVCAR-3nu(A9+), and NIH:OVCAR-4(A9+), were established from drug-refractory patients. 2780ME, 2780CP, OVCAR-3nu(A9+), and OVCAR-4(A9+) are all cross-resistant to irradiation, with D0s of 146, 187, 143, and 203, respectively. However, 2780AD remains sensitive to radiation with a D0 of 111, which is similar to that of A2780 (101). Glutathione (GSH) levels are elevated in 2780ME, 2780CP, OVCAR-3nu(A9+), and OVCAR-4(A9+) to 4.58, 6.13, 12.10, and 15.14 nmol/10^6 cells as compared to A2780, with 1.89 nmol/10^6 cells. However, the GSH level in 2780AD is only minimally higher than that in A2780 (2.94 nmol/10^6 cells). Buthionine sulfoximine, a specific inhibitor of GSH synthesis, significantly increases the radiation sensitivity of 2780ME (changing the D0 from 143 to 95) and 2780AD to a lesser extent, suggesting that intracellular GSH levels may play an important role in the radiation response of certain neoplastic cells. These results suggest that the sequential use of irradiation following chemotherapy with melphalan and cisplatin may be less effective than a combined modality approach, which integrates radiation and chemotherapy prior to the development of drug resistance and cross-resistance to irradiation.

INTRODUCTION

Most patients with ovarian cancer have advanced disease (FIGO Stage III to IV) at the time of diagnosis (10). Combination chemotherapy regimens have produced clinical complete responses in 40 to 50% patients with advanced disease, and second-look laparotomies have demonstrated that the pathological complete response rate is 20 to 30% (5, 14, 44, 46, 47). Whole abdominal irradiation has also been demonstrated to be effective therapy in patients with small volume residual disease (11, 12). In an effort to increase the complete response rate, combined modality approaches with radiation and chemotherapy are currently under investigation at the National Cancer Institute and elsewhere (18, 24, 28). The designs of these clinical trials integrating irradiation and chemotherapy have been empirically derived, and the preliminary results of most studies have failed to demonstrate a marked advantage over the use of combination chemotherapy alone (24, 28). Experimental studies on patterns of radiation and chemotherapeutic sensitivity in relevant model systems of ovarian cancer may be useful in optimizing treatment schedules.

Recently, several ovarian cancer cell lines have been established, both from previously untreated patients and from patients clinically resistant to chemotherapy (2, 22). NIH:OVCAR-4(A9+)2 and NIH:OVCAR-3nu(A9+) were derived from patients who had become refractory to cyclophosphamide, cisplatin, and Adriamycin. In addition, by stepwise incubation of A2780 (a sensitive cell line cloned from an untreated patient) with drugs, variant cell lines with resistance to Adriamycin (2780AD), melphalan (2780ME), or cisplatin (2780CP) were established (16, 22). The sublines are approximately 100-fold, 10-fold, and 10-fold more resistant to the respective drugs than is the parent line (A2780). These cell lines have been used to determine dose-response relationships to chemotherapy, to study the radiosensitivity of human ovarian cancer cells, and to establish the pattern of cross-resistance to irradiation in cell lines with primary resistance induced against antineoplastic drugs. 2780ME is 3-fold more resistant to Adriamycin than is A2780, and 2780AD is 2.5-fold more resistant to melphalan than is the parent line (2). In addition, these cell lines may be useful for evaluating the role of radiation and chemosensitizing agents such as BSO. This drug, a specific inhibitor of glutathione synthesis, has been shown to markedly depress the intracellular GSH level and enhance both radiation and chemosensitivity of many cell types (7, 13, 19–21, 29, 39). The results of these studies may have direct relevance to the design of clinical trials aimed at evaluating combined modality therapy in ovarian cancer.

MATERIALS AND METHODS

Chemicals and Reagents. RPMI 1640, fetal bovine serum, penicillin/streptomycin, and glutamine were obtained from Grand Island Biological

2 The abbreviations used are: NIH:OVCAR-3nu(A9+), a variant of National Institutes of Health Human Ovarian Carcinoma cell line #3 which has been selected for growth in nude mice (nu) and substrate independent growth in agarose (Ag+); NIH:OVCAR-4(A9+), a variant of OVCAR cell line #4 selected for substrate independent growth in agarose; A2780, a human ovarian cancer cell line kindly provided by Dr. S. A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute (16); 2780ME, 2780AD, and 2780CP, variants of A2780 selected for resistance to Adriamycin, melphalan, and cisplatin, respectively; BSO, buthionine sulfoximine; GSH, glutathione; IC50, drug concentration which inhibits 50% colony growth; D0, slope of the exponential portion of the curve.
Characteristics of Ovarian Cancer Cell Lines. By stepwise incubation with individual drugs, variants of a cell line derived from an untreated ovarian cancer patient (A2780) have been developed with resistance to melphalan, Adriamycin, or cisplatin (22). 2780AD was exposed to gradually increasing doses of Adriamycin over a 6-month period up to a maximum dose of 2 \( \mu M \) and then grown in the absence of drug for more than 6 months without loss of resistance to the induction drug. 2780ME and 2780CP were treated similarly with maximum drug concentrations of 10 and 8 \( \mu M \), respectively. The doubling times for the parent line, as well as the variants, are approximately 14 h. Cross-resistance patterns to chemotherapeutic agents have been described previously (2). 2780ME is partially cross-resistant to Adriamycin, and 2780AD is partially cross-resistant to melphalan (2).

NIH:OVCAR-4(A9+) and NIH:OVCAR-3(34+) were obtained from patients who were clinically refractory to cyclophosphamide, Adriamycin, and cisplatin at the time the lines were established (23). The doubling time of NIH:OVCAR-4(A9+) is 25 h, and that of NIH:OVCAR-3(34+) is 63 h. The inherent resistance of NIH:OVCAR-3(34+) and NIH:OVCAR-4(A9+) to melphalan is the same order of magnitude as 2780ME; however, 2780ME is much more resistant to Adriamycin than either NIH:OVCAR-4(A9+) or NIH:OVCAR-3(34+) (Table 1) (19). The IC50 (drug concentration which inhibits 50% colony growth) for melphalan is 5.5 \( \mu M \) in 2780ME as compared to 1.5 \( \mu M \) in NIH:OVCAR-3(34+) and 1.4 \( \mu M \) in NIH:OVCAR-4(A9+). The results for Adriamycin show an IC50 of 1.6 \( \mu M \) in 2780AD versus 0.02 \( \mu M \) and 0.06 \( \mu M \) for NIH:OVCAR-3(34+) and NIH:OVCAR-4(A9+), respectively.

Radiation Studies. Radiation survival curves for A2780 and the variant drug resistant cell lines are shown in Chart 1. The \( D_0 \) for A2780 is 101, while the \( n \) is 1.40. These results are similar to those of other tumor cell lines, both nonhuman and human, reported in the literature (17, 45). 2780ME has a \( D_0 \) of 148 and an \( n \) of 2.12. 2780CP has a \( D_0 \) of 187 and an \( n \) of 1.62. These two lines demonstrate a statistically significant increase in resistance to irradiation. In contrast, 2780AD has a \( D_0 \) of 111 with an \( n \) of 1.48, values which are not statistically different from those of the parent line (A2780).

The radiation survival curves for NIH:OVCAR-4(A9+) and NIH:OVCAR-3(34+) are shown in Chart 2. The \( D_0 \) of 203 and 143 and \( n \)s of 1.08 and 1.53 demonstrate radiation responses similar to 2780ME and 2780CP.

GSH Levels and the Effect of Buthionine Sulfoximine. Total intracellular GSH levels in A2780 and its variants, as well as in NIH:OVCAR-3(34+) and NIH:OVCAR-4(A9+), were determined at the time of irradiation. The results are shown in Table 2. Of A2780 and

### Table 1

<table>
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<th>Cell line</th>
<th>Melphalan</th>
<th>Adriamycin</th>
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Radiotherapy of Human Ovarian Cancer Cells with BSO

**Chart 1.** Radiation survival curves for A2780 and its resistant variants: (O) A2780, (△) 2780AD, (□) 2780ME, and (•) 2780CP. Cells were plated in 0.3% agarose and radiated at doses of 50 to 600 rads. Colonies larger than 60 μm (or 50 cells) were scored on Day 7. Each point represents the mean of at least 4 experiments. Curves were generated using linear regression by least squares analysis. By Student's t test, 2780*° and 2780°CP are statistically different from A2780 (P < 0.01). 2780°CP does not differ significantly from A2780. Bars, SE.

its variants, 2780ME and 2780CP have the highest levels of GSH, with 4.58 and 6.13 nmol/10⁶ cells, respectively. In contrast, 2780AD has a level of 2.94 nmol GSH/10⁶ cells, which is only 50% higher than that of the parent line (1.89 nmol/10⁶ cells). OVCAR-3(Ag+) and OVCAR-4(Ag+) have the highest intracellular GSH levels, with 12.10 and 15.14 nmol/10⁶ cells, respectively.

Preincubation (48 h) with 25 μM BSO in A2780 and its variants (2780CP, 2780AD, and 2780ME) resulted in a decrease of intracellular GSH to less than 0.75 nmol/10⁶ cells (Table 2). The 48-h preincubation with 15 μM BSO in OVCAR-3(Ag+) and OVCAR-4(Ag+) resulted in intracellular GSH levels which were greater than 2.0 nmol/10⁶ cells (Table 2).

**Radiation Sensitization by BSO.** Depletion of intracellular GSH by 48-h monolayer exposure to BSO followed by maintenance of the GSH-depleted state by the presence of BSO in the cloning medium, i.e., during irradiation and for 4 days thereafter, caused a marked sensitization in 2780ME, with the D₀ decreasing from 143 to 95 (Chart 3). Similar treatment of 2780CP resulted in less sensitization to radiation, with the D₀ changing from 183 to 134 (Table 3). However, no significant change in n values was seen for these two lines.

Incubation of OVCAR-3(Ag+) with 15 μM BSO resulted in only a small change in D₀ but a significant change in n. In contrast, BSO treatment of OVCAR-4(Ag+) did not result in radiosensitization (Table 3).

**DISCUSSION**

The development of resistance and cross-resistance to chemotherapy and irradiation is a major clinical problem in the treatment of ovarian cancer. Dose-response relationships and patterns of cross-resistance in established ovarian cancer cell lines...
and their resistant variants may provide clinically important insights into the optimal integration of irradiation and chemotherapy, even though the exact mechanisms of resistance remain to be defined. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters. Since the relationship between in vitro cytotoxicity and clinical response is also undefined, the term resistance may be defined operationally as a change in survival parameters.
study, we have now demonstrated that decreasing the level of intracellular GSH with BSO can completely restore radiation sensitivity to 2780CP. Despite GSH levels which were decreased to the same extent as in 2780ME (<0.75 nmol/10^6 cells), BSO treatment in 2780CP caused less sensitization to irradiation. This suggests the elevation of intracellular GSH may not be the only mechanism involved in the development of cross-resistance to irradiation in cells with primary resistance induced to cisplatin, in contrast to the apparent situation in which primary resistance is induced with melphalan.

Because of the increased cytotoxicity of BSO in the OVCAR cell lines, OVCAR-3(AgM) and OVCAR-4(AgM) were treated with 15 μM BSO. At this dose level, total GSH could not be depressed below 2.0 nmol/10^6 cells (a level higher than the inherent level of A2780). The observation that BSO treatment had no effect on the radiation survival parameters of OVCAR-3(AgM) and OVCAR-4(AgM) suggests that there may be a threshold below which GSH levels must be depressed in order to produce radiation sensitization.

While it is clear that cells with elevated intracellular GSH levels are more resistant to ionizing radiation and that reduction of GSH levels (via specific enzyme inhibition) can lead to reversal of that resistance, the molecular mechanisms involved have not been established. Possible explanations for GSH protection from radiation-induced damage include the immediate reduction of free radicals both on DNA itself and on other radiolytic products, as well as modifications of DNA repair enzymes or their environment to enhance activity. Studies to evaluate these possibilities as they relate to both radiation and chemotherapeutic drug resistance are in progress.

REFERENCES


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