Cross-Resistance to Diverse Drugs Is Associated with Primary Cisplatin Resistance in Ovarian Cancer Cell Lines

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ABSTRACT

We have previously obtained, by exposure to near continuous increasing concentrations of cisplatin, a panel of human ovarian cancer cell lines that exhibit a wide range of primary resistance to the drug (9- to >400-fold). These cells had strikingly increased (4- to 50-fold) levels of glutathione (GSH) as compared with the drug-sensitive cells of origin (A. K. Godwin et al., Proc. Natl. Acad. Sci. USA, 89: 3070-3074, 1992). Utilizing this panel of resistant cell lines, we evaluated cross-resistance to classical alkylating agents, natural product drugs, and irradiation. We observed that cross-resistance to carboplatin paralleled that of cisplatin, culminating in approximately 250-fold resistance. Similarly, melphalan cross-resistance continued to increase to >400-fold and again paralleled the primary cisplatin resistance. Cell lines with low to very high levels of resistance to cisplatin are 8- to 850-fold resistant to the epipodophyllotoxin derivative etoposide. Cross-resistance is also observed for other natural product drugs, including Adriamycin (~80-fold), mitoxantrone (~440-fold), and taxol (~40-fold). Cross-resistance to irradiation is, however, modest (<2-fold). The cells with the greatest primary resistance to cisplatin most commonly had the highest cross-resistance to the other drugs examined. The cross-resistance to the natural product category drugs was found not to be mediated by the products of either the multidrug resistance 1 (MDR1) or multidrug resistance-associated protein (MRP) genes based on lack of coordinate increased expression or amplification of these genes as assessed by Northern and Southern blot analyses. Furthermore, verapamil failed to markedly increase drug sensitivity. Although there was no indication that these natural product drug efflux pumps were operative, we observed decreased doxorubicin accumulation in these cell lines cross-resistant to natural products. In addition, alternations in DNA topoisomerase II mRNA levels, which have been observed in a variety of human tumor cell lines selected in vitro for resistance to etoposide or teniposide, were not detected. Only intracellular levels of GSH correlated with cross-resistance to these diverse anticancer agents and partial loss of resistance was associated with a marked decrease in glutathione levels. In the absence of alternative mechanisms, we speculate that the very broad clinically relevant cross-resistance seen in this model system may, at least in part, be the direct result of GSH-mediated drug inactivation or may be due to a combination of GSH conjugation to drug and conjugate efflux mediated by the putative ATP-dependent glutathione S-conjugate export pump.

INTRODUCTION

Treatment of ovarian cancer with cisplatin-based combination chemotherapy has yielded increased complete response rates, and cisplatin has become a cornerstone in the treatment of this disease during the last two decades (1, 2). Nevertheless, an improvement in long-term survival of cisplatin-treated patients with advanced stage ovarian cancer has appeared to plateau over the last decade, since the majority of these patients relapse after responding to initial chemotherapy and die of their chemotherapy-refractory disease (3). Thus, emergence of drug-resistant tumor cells leads to tumor progression and subsequent death. The resistance observed clinically is quite complex in that, in addition to resistance to the drugs used for primary chemotherapy, tumors are refractory to diverse unrelated drugs. Hence, second line chemotherapy has been of limited benefit in ovarian cancer (2). The one potential exception to this complex pattern of clinical multidrug resistance is the recently observed utility of taxol as a salvage therapy in refractory ovarian cancer (4).

One approach, in addition to new drug discovery, with the potential to improve the treatment for ovarian cancer is to identify the mechanisms responsible for the complex resistance phenotype observed clinically and then exploit pharmacological approaches to prevent the emergence of drug resistance or reverse the phenotype when it occurs. Resistance to the diverse drugs generally categorized as natural products has been most studied. Increased expression of the MDR1 gene which encodes the p170 glycoprotein is one mechanism by which cells may become resistant to natural product drugs (5–7). Two other proteins potentially involved in natural product resistance have more recently been reported: the multidrug resistance-associated protein (MRP); and a protein identified using an antibody that recognizes a conserved ATP-binding domain in mitoxantrone-resistant cell lines (8, 9). In addition, alternations in DNA topoisomerase II have been identified in a cell line selected for resistance to natural products including etoposide, mitoxantrone, Adriamycin, or teniposide (10–14). It is of note that the activity of classical alkylating agents and platinum drugs, which are major components in the chemotherapy of ovarian cancer, have been found not to be influenced by these mechanisms of resistance where studied. This is of particular interest since the failure of primary chemotherapy with these agents results in tumors refractory to second line therapy with the diverse natural products as well as other DNA-reactive drugs. This suggests the existence of a mechanism or mechanisms which contribute to primary resistance and also to the broad cross-resistance typical of clinical ovarian cancer.

Resistance to platinum drugs and alkylating agents is multifactorial including as components: decreased drug accumulation (non-MDR1 mediated); increased cellular inactivation; and increased DNA repair. Of these mechanisms, increased drug inactivation, mediated by glutathione, is a mechanism with the potential to contribute to both primary resistance and the complex drug-resistant phenotype observed in ovarian cancer and other solid tumors similarly treated. Glutathione is the predominant intracellular nonprotein thiol in all mammalian cells and plays an important role in homeostasis and likely xenobiotic detoxification. The concept that the binding of GSH to anticancer drugs could serve as a means of their inactivation and hence contribute to drug resistance may be credited to Meister (15). The experimental support for the validity of this hypothesis, although not conclusive, is extensive. We and others have shown in numerous cell lines that selection for cisplatin and alkylating agent resistance is accompanied by elevations in GSH and that depletion of GSH by nutritional deprivation or treatment with buthionine sulfoximine, an irreversible inactivator of GSH synthesis, reversed primary and secondary resistance to cisplatin (16). An additional mechanism by which GSH could participate in the development of resistance is by its role in the detoxification of drugs that are bound to it. For instance, GSH has been shown to participate in the detoxification of etoposide (17). In another study, we have shown that GSH conjugates of cisplatin are more stable than the cisplatin monoglutathione adducts in circulation, and that GSH conjugates of cisplatin are preferentially secreted into the bile (18). These studies support the idea that GSH may play a role in the development of drug resistance through the binding and detoxification of drugs that are bound to it (15).
The C-series were exposed to cisplatin for 72 h once every 10-21 days. C30R
(21). All cell lines were maintained as monolayers in RPMI 1640 containing
exposure to increasing concentrations of cisplatin in the case of the C series
patient (26). A panel of platinum-resistant cell lines derived from A2780
(Grand Island, NY). All other chemicals and reagents were obtained from
was obtained from Eli Lilly. Glutamine-free concentrated RPMI 1640, fetal
portive of a role for GSH in resistance to platinum-containing drugs
which have shown a strong correlation in cell lines (21-23) and
clinical samples (24, 25) between GSH amount and level of resistance.
Although it is unlikely that GSH is the sole contributor to cisplatin and
roles for MDR1, MRP, and topoisomerase II expression in manifestation of the com-
plex cross-resistance phenotype typical of ovarian cancer.

In the present work, we determined the cross-resistance phenotype
of a related series of human ovarian cancer cell lines selected in vitro
for a wide range of primary cisplatin resistance. Roles for MDR1,
MRP, and topoisomerase II expression in manifestation of the com-
plex cross-resistance phenotype were excluded.

MATERIALS AND METHODS

Chemicals and Reagents. Cisplatin, carboplatin, and VP-16 were obtained
from Bristol Myers (Evansville, IN). Melphalan was obtained from Sigma
Chemical Co. (St. Louis, MO). Adriamycin was obtained from Cetus Corp.
(Emeryville, CA). Mitoxantrone was obtained from the American Cyanamid
Company (Pearl River, NY). Taxol for clinical use was provided by the
Division of Cancer Treatment, National Cancer Institute, NIH, and was resus-
pended at a concentration of 6 mg/ml in 50% polyoxyethylated castor oil
grade 480; the specific activity, 50-60 mCi/mmol) was obtained from the
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Influx and Efflux of Doxorubicin. Cells were harvested with trypsin and
plated as a monolayer in 35-mm dishes at a concentration of 5 X 10^5 cells/dish.
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Cytotoxicity Assays. Cisplatin, carboplatin, melphalan, Adriamycin, mit-
oxantrone, taxol, and etoposide cytotoxicity was determined by the tetra-
zolium assay (27). Cells (1000 to 8000) were plated in 150 μl of medium/Well in
96-well plates (Corning Co., Corning, NY). After incubation overnight, each
drug was added in varying concentrations. After 72 h, 40 μl of 5 mg/ml
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Values are means ± SD of triplicate determinations obtained from at least 2 independent experiments.

<table>
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<tr>
<th>Drugs</th>
<th>IC_{50} for A2780(μM)</th>
<th>CP20</th>
<th>CP70</th>
<th>C50</th>
<th>C60</th>
<th>C100</th>
<th>C200</th>
</tr>
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<tbody>
<tr>
<td>CDDP</td>
<td>0.347 ± 0.004*</td>
<td>5</td>
<td>17</td>
<td>290</td>
<td>255</td>
<td>412</td>
<td>364</td>
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<tr>
<td>CBDCA</td>
<td>0.053 ± 0.763</td>
<td>21</td>
<td>24</td>
<td>141</td>
<td>166</td>
<td>154</td>
<td>199</td>
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<tr>
<td>L-PAM</td>
<td>0.767 ± 0.111</td>
<td>12</td>
<td>14</td>
<td>138</td>
<td>120</td>
<td>234</td>
<td>346</td>
</tr>
<tr>
<td>ADR</td>
<td>0.048 ± 0.000</td>
<td>3</td>
<td>6</td>
<td>22</td>
<td>30</td>
<td>36</td>
<td>76</td>
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<tr>
<td>MIT</td>
<td>0.013 ± 0.0001</td>
<td>2</td>
<td>8</td>
<td>35</td>
<td>261</td>
<td>346</td>
<td>415</td>
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<tr>
<td>VP-16</td>
<td>0.054 ± 0.002</td>
<td>6</td>
<td>19</td>
<td>222</td>
<td>296</td>
<td>370</td>
<td>833</td>
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<tr>
<td>Taxol</td>
<td>0.0025 ± 0.063</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>26</td>
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GSH (nmol/10^6 cells) for A2780

<table>
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<tr>
<th></th>
<th>Relative ratio</th>
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<tr>
<td>1.707 ± 0.140</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
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<tr>
<td>13</td>
<td>19</td>
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<td>25</td>
<td>24</td>
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<td>42</td>
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a IC_{50} determined by MTT assay continuously exposed for 72 h. Cross-resistance is defined as the ratio of the IC_{50} of the resistant variant to the relatively sensitive parental A2780
cell line. Values are means ± SD of triplicate determinations obtained from at least 2 independent experiments.

b IC_{50} determined by MTT assay continuously exposed for 72 h. Cross-resistance is defined as the ratio of the IC_{50} of the resistant variant to the relatively sensitive parental A2780
cell line. Values are means ± SD of triplicate determinations obtained from at least 2 independent experiments.
kit (Stratagene) to a specific activity of $>5 \times 10^6$ dpm/µg. The following cDNA probes used were kindly provided by the following investigators: A. Fojo, p5A5/MDR [1.4-kbp human MDR1 fragment (29)]; G. Kruh (a 1.2-kbp human MRP fragment; Fox Chase Cancer Center); L. Liu, pC15 [a 1.8-kbp human p170 topoisomerase II fragment (30)] and pGCS19-1 [a 3.7-kbp human full-length γ-glutamylcysteine synthetase cDNA fragment; described by us, (21)]. Autoradiography was performed at -70°C for 1 to 4 days following washing.

Glutathione Determinations. Intracellular GSH levels were determined as described previously (21).

RESULTS

Multidrug Sensitivity of Cisplatin-resistant Cells to Various Anticancer Agents. The relative sensitivity to melphalan, a classical alkylating agent, to carboplatin, the currently widely used cisplatin analogue, to irradiation, and to the diverse natural products Adriamycin, mitoxantrone, VP-16, and taxol, was evaluated in a panel of related human ovarian cancer cell lines selected in vitro for a range of primary cisplatin resistance (Table 1). As shown in Fig. 1, cross-resistance to carboplatin paralleled that of cisplatin culminating in approximately 250-fold resistance in C200 cells; these cells are approximately 400-fold more resistant to cisplatin than is the parental A2780 cell line. Similarly, melphalan cross-resistance continued to increase to >400-fold and again paralleled the primary cisplatin resistance. Cell lines with low to very high levels of resistance to cisplatin (9- to >400-fold) are 8- to 850-fold resistant to VP-16, which is usually included among the diverse drugs considered to be part of the classical multidrug resistance (MDR) phenotype. Cross-resistance was also observed for other natural product category drugs, including Adriamycin (nearly 80-fold), mitoxantrone (up to 440-fold), and taxol. Cross-resistance to taxol, a unique antineoplastic agent which
appears to exert its cytotoxic action as a result of interference with microtubular structure and function, had an interesting pattern. CP20, CP70, and C30 cells with 9-, 29-, and 250-fold resistance to cisplatin, respectively, were not cross-resistant to taxol (Table 1; Fig. 1). The more highly platinum-resistant variants (C100 and C200 cells), however, displayed significant levels of cross-resistance to taxol (~40-fold). In contrast to marked cross-resistance to diverse drugs observed in these cell lines, cross-resistance to irradiation was not remarkable (~2-fold) as measured by $D_0$ values (A2780, 155, CP70, 270, C100, 200, C200, 205).

Expression of the MDR1, MRP, Topoisomerase II, and γ-GCS Genes. In order to determine whether known mechanisms of resistance to diverse natural products were operative in manifestation of the cross-resistance phenotype of these cells, we evaluated the expression of three genes, MDR1, MRP, and topoisomerase II. Overexpression of the transmembrane transport protein P-glycoprotein has been detected in many multidrug-resistant cell lines and in a variety of tumors from cancer patients. This protein is encoded by the MDR1 gene, and in vitro studies have shown that it confers resistance to a range of natural products that are used as chemotherapeutic drugs (29). Using Northern blot analysis, the drug-resistant cell lines were examined for the presence of mRNA coding for the P-glycoprotein. As indicated in Fig. 2, none of the resistant cell lines expressed detectable levels of MDR1 mRNA, while MDR1 mRNA was readily detectable in the colon carcinoma cell line HCT-15 included as a positive control.

To evaluate alternative pathways involved in the observed multidrug-resistant phenotype, we examined the cell lines listed in Table 1 for altered expression of the MRP and topoisomerase II genes. MRP is a newly identified member of the ATP-binding cassette transmembrane transporter superfamily the overexpression of which is potentially associated with multidrug resistance in human lung cancer cell lines and is distantly related to MDR1. As shown in Fig. 2, MRP mRNA levels varied only slightly in the drug-resistant variants as compared to parental A2780 cells. In fact, most of the resistant variant displayed lower steady-state mRNA levels (~2-fold) as compared to A2780 and HCT15 cells. Previous studies have shown that resistance to cisplatin, doxorubicin, and VP-16 may be associated with alterations in topoisomerase II expression and activity. Since the platinum-resistant variants are highly cross-resistant to these anticancer agents, topoisomerase II mRNA levels were also evaluated. As shown in Fig. 2, there was no apparent difference in mRNA levels between drug-sensitive A2780 cells and the resistant sublines. We next examined message levels for γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis, where we observed dramatic increases in C100 and C200 cells, comparably lower levels were observed in cell lines CP20, CP70, HCT15, and parental A2780. These cell lines were also examined for amplification of the MDR1, MRP, topoisomerase II, and γ-GCS genes by Southern blot analysis which revealed no change in gene copy number.

Doxorubicin Accumulation. In order to determine whether non-MDR1-mediated decreased drug accumulation contributed to the broad spectrum of cross-resistance to natural product drugs displayed in these cell lines, the accumulation of doxorubicin was determined in A2780, CP70, C30, C100, and C200 cells. As shown in Fig. 3A, there was an approximately 2-fold reduced accumulation of [14C]doxorubicin after 4 h of drug exposure in the most resistant variant (i.e., C200 cells) as compared to drug-sensitive A2780 cells. The efflux of drug from A2780 and C200 cells loaded with [14C]doxorubicin by 4 h exposure was also determined (Fig. 3B). There was some loss of intracellular radioactivity from C200 cells as compared to the parental A2780 cells. Approximately 20% of radioactive doxorubicin effluxed from C200 cells after 90 min, while nearly all the radiolabeled drug remained in A2780 cells.

Effect of Verapamil on Drug Cytotoxicity. To further exclude the possible involvement of MDR1 and related genes in the natural product cross-resistance phenotype of these cells, we evaluated the effect on cytotoxicity of blocking this pump with verapamil. Incubation of A2780, C30, and C200 with 5 μM verapamil did not significantly alter their sensitivity mitoxantrone, VP-16, or melphalan (Table 2). In contrast, verapamil had a noticeable effect on mitoxantrone cytotoxicity in 2780 AD10 cells, where an ~5-fold shift in IC50 was observed. [AD10 cells were selected for primary resistance to Adriamycin (31) and have previously been shown to overexpress the MDR1 gene product].

Correlation between Drug Resistance and Intracellular GSH Levels. We have previously reported a strong correlation between glutathione, the most abundant nonprotein thiol in cells, and the primary cisplatin resistance of these cell lines (21). Based on these studies and the studies of others showing a role for glutathione in resistance to several drugs, we sought to determine if a linear relationship was present between cross-resistance to the diverse drugs reported here and cellular glutathione. The concentration of GSH in these cell lines is shown in Table 1. A strong to moderate correlation was observed between intracellular levels of GSH and IC50 obtained after 72 h continuous exposure to cisplatin ($r = 0.94$), carboplatin ($r = 0.83$), melphalan ($r = 0.89$), Adriamycin ($r = 0.80$), mitoxan-
trone (r = 0.81), and etoposide (r = 0.78) (Fig. 4). Furthermore, highly drug-resistant variants maintained in a drug-free environment (i.e., without cisplatin) for long periods of time (i.e., at least 2 years) showed increased sensitivity to cisplatin. The levels of GSH in C30R (i.e., without cisplatin) for long periods of time (i.e., at least 2 years) showed increased sensitivity to cisplatin. The levels of GSH in C30R, (r = 0.81), and etoposide (r = 0.78) (Fig. 4). Furthermore, high drug-resistant variants maintained in a drug-free environment (i.e., without cisplatin) for long periods of time (i.e., at least 2 years) showed increased sensitivity to cisplatin. The levels of GSH in C30R, and C200R, partial revertants, were one-half and one-fourth of that found in C30 and C200 cells (Table 3), which further substantiates the role of increased cellular glutathione in the drug resistance phenotype.

**DISCUSSION**

We have exposed the A2780 human ovarian cancer cell line to increasing concentrations of cisplatin to isolate a series of related cell lines which vary widely in their primary cisplatin resistance. Even in this relatively simple model system, it is apparent that primary cisplatin resistance is associated with the development of numerous resistance mechanisms including: decreased cisplatin accumulation; increased potential for inactivation by increased amounts of glutathione; increased ability to repair total platinum-DNA lesions; alterations in the categories of lesions formed at the DNA sequence level; and increased ability to repair cisplatin interstrand cross-links in specific DNA sequences (32). The cell lines described in our study will complement those developed by other investigators with lower levels of cisplatin resistance (22, 23, 33, 34). It is evident that our cell lines are well suited to defining the fullest possible repertoire of mechanisms which can contribute to cisplatin resistance. The use of these and of other related cell lines which vary widely in resistance will allow determination of the relative significance of individual mechanisms as cisplatin resistance increases. It will then be necessary to determine the relative significance of these individual mechanisms in clinical cancer specimens.

We have now examined the cell lines for cross-resistance to diverse chemotherapeutic agents to determine whether the characteristics of these cells are consistent with the drug resistance phenotype of clinical ovarian cancer. It is well accepted that ovarian tumors that become refractory to aggressive cisplatin-based chemotherapy are unlikely to respond to the diverse other drugs in the arsenal of chemotherapeutic agents. The emerging exception to this rule are data on responses to taxol (4, 35, 36). In our model system we observed strikingly high levels of cross-resistance to carboplatin, melphalan, etoposide, Adriamycin, and mitoxantrone (440-fold changes in sensitivity, respectively). These changes paralleled the primary cisplatin resistance. The exception to these findings was with regard to taxol cross-resistance which was apparent only in the more highly resistant variants and only reached 38-fold. This information is consistent with emerging clinical data on taxol as noted above. Hence, the cross-resistance profile of these cell lines mimics cisplatin-refractory clinical ovarian cancer and suggests that this model system should yield important leads as to the mechanisms responsible for the clinical cross-resistance which is observed after the failure of cisplatin therapy.

In order to gain insight into the issue of whether the cross-resistance to the natural product drugs we observed could be mediated by previously described mechanisms, we examined the expression of three genes, MDR1, MRP, and topoisomerase II. Functional assays in the form of drug accumulation, and pharmacological manipulation of natural product cytotoxicity were also performed in order to provide additional evidence of whether the classical p170 glycoprotein drug efflux pump, coded by the MDR1 gene, was operative as a mechanism of natural product resistance in this model system. There was no evidence of increased steady-state expression of the MDR1 gene or the additional evidence of whether the classical p170 glycoprotein drug resistance mechanisms as cisplatin resistance increases. It will then be necessary to determine the relative significance of these individual mechanisms in clinical cancer specimens.

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Fig. 4. Correlation between intracellular GSH and fold resistance of cisplatin-resistant ovarian cancer cell lines to 6 drugs. Fold resistance was defined as the ratio of the IC50 of the resistant variant to the relatively sensitive parental A2780 cell line. Intracellular GSH levels were determined as described in "Materials and Methods."
Cross-resistance to etoposide was remarkable and, in fact, substantially exceeded primary cisplatin resistance. In the absence of MDR1-mediated cross-resistance to this natural product, we evaluated the possibility of altered expression of topoisomerase II, the target for etoposide. Reports by others have shown that etoposide resistance can be associated with similar or decreased levels of its target enzyme (11, 37). We, however, observed no relationship between topoisomerase II expression and etoposide sensitivity. This does not exclude the possibility that etoposide resistance results from a difference in activity of the topoisomerase II enzyme in the resistant variants relative to the parental cell line.

We and others have reported a close association between platinum and classical alkylating agent resistance and cellular glutathione in various model systems. We have shown that the increased glutathione levels are associated with increased expression of γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase (Fig. 2; Ref. 21). Furthermore, reversion to cisplatin, Adriamycin, and VP-16 sensitivity was associated with decreased levels of intracellular glutathione (Table 3 and other data not included). We evaluated whether a linear relationship exists between GSH and the sensitivity to the diverse drugs described here. As would be anticipated based on the strong relationship between the level of primary cisplatin resistance and cross-resistance to individual drugs, we found a moderate to strong correlation (r = 0.785 to 0.891) between cross-resistance to individual drugs and glutathione. It is possible that the conjugation of GSH to drug is sufficient to abrogate cytotoxicity. The tantalizing proposal, however, has been put forward by others (38) that these conjugates could act in some cases as cytotoxic intermediates. Therefore, resistance would require the excrcion of the conjugate by a molecular pump (e.g., the putative ATP-dependent glutathione S-conjugate export pump). Ongoing investigations include transfection of the parental A2780 cells with cDNAs for γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase under the control of strong promoters in order to markedly increase glutathione levels in the absence of other potential contributors to resistance. Evaluation of sensitivity to the diverse drugs examined here in these transfected cells will clarify the level to which glutathione contributes to the complex clinically relevant cross-resistance we have observed. Furthermore, application of multiple molecular biological approaches to this model has the potential to uncover additional mechanisms responsible for the phenotype.

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REFERENCES

7. Roninson, I. B. Cross-resistance to etoposide was remarkable and, in fact, substantially exceeded primary cisplatin resistance. In the absence of MDR1-mediated cross-resistance to this natural product, we evaluated the possibility of altered expression of topoisomerase II, the target for etoposide. Reports by others have shown that etoposide resistance can be associated with similar or decreased levels of its target enzyme (11, 37). We, however, observed no relationship between topoisomerase II expression and etoposide sensitivity. This does not exclude the possibility that etoposide resistance results from a difference in activity of the topoisomerase II enzyme in the resistant variants relative to the parental cell line.

We and others have reported a close association between platinum and classical alkylating agent resistance and cellular glutathione in various model systems. We have shown that the increased glutathione levels are associated with increased expression of γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase (Fig. 2; Ref. 21). Furthermore, reversion to cisplatin, Adriamycin, and VP-16 sensitivity was associated with decreased levels of intracellular glutathione (Table 3 and other data not included). We evaluated whether a linear relationship exists between GSH and the sensitivity to the diverse drugs described here. As would be anticipated based on the strong relationship between the level of primary cisplatin resistance and cross-resistance to individual drugs, we found a moderate to strong correlation (r = 0.785 to 0.891) between cross-resistance to individual drugs and glutathione. It is possible that the conjugation of GSH to drug is sufficient to abrogate cytotoxicity. The tantalizing proposal, however, has been put forward by others (38) that these conjugates could act in some cases as cytotoxic intermediates. Therefore, resistance would require the excrcion of the conjugate by a molecular pump (e.g., the putative ATP-dependent glutathione S-conjugate export pump). Ongoing investigations include transfection of the parental A2780 cells with cDNAs for γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase under the control of strong promoters in order to markedly increase glutathione levels in the absence of other potential contributors to resistance. Evaluation of sensitivity to the diverse drugs examined here in these transfected cells will clarify the level to which glutathione contributes to the complex clinically relevant cross-resistance we have observed. Furthermore, application of multiple molecular biological approaches to this model has the potential to uncover additional mechanisms responsible for the phenotype.

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