

# Characterization of a *cis*-Diamminedichloroplatinum(II)-resistant Human Ovarian Cancer Cell Line and Its Use in Evaluation of Platinum Analogues<sup>1</sup>

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## ABSTRACT

Human ovarian cancer cell lines with stable cisplatin resistance have been developed by chronic exposure of the parent cisplatin-sensitive A2780 line to increasing concentrations of cisplatin. 2780<sup>CP8</sup> (CP8 refers to this cell line's growth in medium containing 8  $\mu$ M cisplatin) has several clonal cytogenetic abnormalities but lacks homogeneously staining regions or double-minute chromosomes. It has a significantly greater monolayer growth rate, cloning efficiency in agarose, and total glutathione content compared to the A2780 line, but similar activities of several glutathione-dependent enzymes. The 2780<sup>CP8</sup> subline is 7.3-fold resistant to cisplatin compared to the A2780 line, as well as cross-resistant to irradiation and melphalan. It is not cross-resistant to Adriamycin, but this develops with increased cisplatin resistance (14-fold) obtained by further cisplatin exposure of 2780<sup>CP8</sup>. Of the cisplatin analogues tested which are of current clinical interest, carboplatin, iproplatin, and tetraplatin, only the latter is more cytotoxic than cisplatin in the A2780 and 2780<sup>CP8</sup> lines. The 2780<sup>CP8</sup> subline is also cross-resistant to these analogues in the relative order carboplatin > iproplatin > tetraplatin (most to least cross-resistant). Treatment of a highly cisplatin resistant cell line (2780<sup>CP8</sup>) with either melphalan or cisplatin was associated with a significant increase in [<sup>3</sup>H]thymidine incorporation into DNA in the presence of 10 mM hydroxyurea compared with the parent sensitive cell line which showed essentially no capacity to repair DNA damage by these drugs. A2780 and its cisplatin-resistant cell lines may thus be useful in studying drug resistance mechanisms, in screening new drugs for activity (especially against drug resistant tumors), and in formulating induction and salvage therapies for ovarian cancer.

## INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II)], a clinically important antineoplastic agent with activity against a wide spectrum of tumors (2, 3), has been particularly useful for the treatment of testicular and ovarian cancer. The cure rate for patients with advanced testicular cancer has increased from 30-40% achievable with non-cisplatin-based therapy to 70-80% with cisplatin-based combination regimens (2, 4). In patients with advanced epithelial ovarian cancer cisplatin-based regimens have produced increased complete response rates, and in some studies prolonged overall survival compared to single-agent therapy (5, 6). However, the majority of patients with bulky ovarian cancer are not cured with standard cisplatin regimens primarily due to the development of acquired drug resistance (7). In addition, while the dose of cisplatin is a critical factor in achieving optimal results in ovarian cancer patients (8), the toxicity of cisplatin has prevented administration of doses greater than 200 mg/m<sup>2</sup> per cycle even when cisplatin is

administered in hypertonic saline with vigorous chloruresis (9).

In an effort to improve the therapeutic index for cisplatin, several hundred analogues of cisplatin have undergone initial preclinical evaluation (10). Alterations in the chloride leaving groups have led to analogues which have the same spectrum of activity as cisplatin but which may be less toxic and consequently permit further escalations of dose (11). Other analogues have been developed by altering the nature of the nonexchangeable amino ligands in an effort to develop analogues non-cross-resistant with cisplatin for use in patients with cisplatin-resistant tumors (3, 12).

Experimental studies using relevant *in vitro* and *in vivo* models of drug-resistant and drug-sensitive human ovarian cancer may lead to the improved use of cisplatin and its analogues (13). The *in vitro* patterns of cross-resistance in such matched ovarian cancer cell lines may help identify those analogues of potential utility in the treatment of cisplatin-resistant tumors. In addition, the nature of the dose-response relationship for cisplatin and its analogues is important for the design of high dose chemotherapy studies.

Cisplatin-sensitive and -resistant cell lines may also be useful in unraveling the molecular mechanism(s) of cisplatin cytotoxicity. While the primary lesion responsible for the cytotoxicity of cisplatin is likely a result of its reaction with DNA (14, 15), the exact nature of the critical cytotoxic lesion(s) has not been established. Interstrand DNA-DNA and DNA-protein as well as intrastrand cross-links have been identified (16) although the interstrand cross-links (detected by alkaline elution) account for only 1% of the total platinum bound to DNA and thus may be insufficient to fully account for all of the antitumor effects of cisplatin. A major adduct of cisplatin is the intrastrand N7d(GpG)-diammine platinum adduct which comprises 40-60% of the platinum bound to DNA (17). The X-ray structure of such an intrastrand bidentate adduct on adjacent deoxyguanosines has recently been characterized (18). Measurement of the intrastrand N7d(GpG)-diammine platinum adduct using a specific enzyme-linked immunosorbent assay as well as of interstrand cross-links with alkaline elution assays in cisplatin-sensitive and -resistant cell lines may provide insights regarding the importance of these lesions in the cytotoxicity of platinum drugs (19). In addition, an improved understanding of the mechanisms responsible for cisplatin resistance may lead to biochemical techniques or pharmacological strategies by which this resistance may be diminished or circumvented as well as to the design of specific analogues less cross-resistant with cisplatin.

In this report we describe the characteristics of a cisplatin-resistant human ovarian cancer cell line which was developed from a cisplatin-sensitive parent cell line. The patterns of cross-resistance to other antineoplastic drugs and to irradiation indicate that the cell line may be of potential use in studying the mechanisms of primary cisplatin resistance as well as of cross-resistance to other drugs. We have examined these cell lines for their capacity to respond to cisplatin and melphalan damage by initiation of unscheduled DNA synthesis as an indicator of

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<sup>1</sup>These data were presented, in part, at the 76th Annual Meeting of the American Association for Cancer Research, Houston, TX (1), and the 77th Annual Meeting, Los Angeles, CA.

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repair capacity. In addition, we have used this matched pair of drug-sensitive and drug-resistant cell lines to evaluate the relative activity and cross-resistance of cisplatin and several of its analogues which are currently of clinical interest.

## MATERIALS AND METHODS

**Supplies.** Crystalline U-100 insulin was obtained from Eli Lilly and Co., Indianapolis, IN. Glutamine-free concentrated RPMI 1640 medium and all other components of complete tissue culture medium (termed "complete medium" hereafter) were from Grand Island Biological Co., Grand Island, NY, as was Eagle's minimal essential medium. Cisplatin analogues NSC-241240 (carboplatin), NSC-256927 (ipropilatin), and NSC-363812D (tetraplatin), and melphalan were provided by the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. [<sup>3</sup>H]dThd<sup>4</sup> (50–80 Ci/mmol) and Aquassure were from New England Nuclear, Boston, MA. All other chemicals and biochemicals were purchased from Sigma Chemical Co., St. Louis, MO.

**Cell Lines and Tissue Culture.** The A2780 human ovarian cancer cell line, established from tissue obtained from an untreated patient, was kindly provided by Dr. S. Aaronson of the National Cancer Institute. Sublines which survive intermittent exposure to 8, 20, or 70 μM cisplatin in monolayer culture (designated as 2780<sup>CP8</sup>, 2780<sup>CP20</sup>, and 2780<sup>CP70</sup>, respectively) were obtained by exposure of the A2780 line to stepwise-increasing cisplatin concentrations. Initial cisplatin exposure was at a concentration of 3 nM, with the cell line exposed three times for 3-day periods during a 3–6-week period allowing for growth recovery between cycles. After the completion of three cycles of drug, the dose was doubled and the procedure repeated until the noted drug levels were achieved. All cell lines were maintained as monolayers in drug-free complete medium [RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 μM L-glutamine, insulin (0.25 units/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml)]. The cisplatin-resistant cell lines were used in the experiments described below 1–6 mo after continuous growth in media without cisplatin.

**Cytotoxicity Assays.** Clonogenic cell survival following treatment with platinum compounds or irradiation was assessed in a two-layer soft agarose system, as previously described (20, 21). Briefly, single cell suspensions were first prepared from exponentially growing monolayer cultures (4 days after subculture) by brief exposure to 0.05% trypsin/0.02% EDTA (both w/v in phosphate buffered saline) followed by gentle passage through 22-gauge needles. Suspensions were then adjusted to standard cell concentrations using an electronic particle counter (model ZBI; Coulter Electronics, Hialeah, FL). Cells were then mixed with agarose in complete medium (0.3%, w/v, final concentration) and 1-ml aliquots (containing 10,000 cells) overlaid on chilled 1-ml base layers of 0.6% agarose in complete medium contained in 35-mm diameter plastic dishes (3035 tissue culture dishes; Costar, Cambridge, MA). Top agarose layers also contained freshly prepared drugs or an equal volume of diluent (normal saline) in studies of continuous drug exposure.

**Karyotypic Analysis.** Chromosome preparations were examined after both conventional Giemsa staining and trypsin-Giemsa banding and results were reported using standard nomenclature, as described previously (22).

**Other Assays.** The A2780 and the cisplatin-resistant cell lines were plated in multiple flasks at a density of  $1.5 \times 10^6$  cells/75-cm<sup>2</sup> tissue culture flask. Tissue culture media was changed on days 1, 4, and 7 after plating. Cell suspensions were then prepared, as described above, at various times after plating. A portion of each suspension was used to measure total GSH (GSH + GSH disulfide) content using a modification of the method of Suzukake *et al.* (23) in which the rate (rather than the total extent) of 2-nitro-5-thiobenzoic acid formation was monitored spectrophotometrically. The initial velocity of this reaction is linearly related to total GSH content over sample concentrations of 2–80 nmol/ml (data not shown).

<sup>4</sup> The abbreviations used are: dThd, thymidine; CRI, cross-resistance index; GSH, glutathione; IC<sub>50</sub>, drug concentration causing 50% reduction in surviving fraction; UDS, unscheduled DNA synthesis.

**Statistical Analysis.** Differences between means were tested using the unpaired two-tailed Student's *t*-test, and linear regression analysis was performed by the least-squares method (24).

**Measurement of UDS.** The cell lines were screened for their capacity to perform UDS with exposure to melphalan or cisplatin. In the absence of semiconservative DNA synthesis [<sup>3</sup>H]dThd incorporation into acid-precipitable DNA is taken as a measure of repair of induced damage to DNA (25). As these carcinoma cell lines show little propensity toward contact-associated inhibition of cell growth and [<sup>3</sup>H]dThd incorporation into DNA does not discriminate between DNA replication and unscheduled DNA synthesis (26), special care was necessary to limit semiconservative synthesis during the assay of these cells. Growth of cells to confluence in 25-cm<sup>2</sup> culture dishes using Eagle's minimal essential medium containing 10% (v/v) fetal bovine serum followed by maintenance of cultures for 3 days in Eagle's minimal essential medium deficient in arginine but with L-glutamine and 2.5% (v/v) dialyzed fetal bovine serum inhibited further growth of A2780 and the resistant variants by ≥99%. At this point, to further inhibit semiconservative DNA synthesis, hydroxyurea (10 mM) was added to each dish, and after 1 h, drug (melphalan or cisplatin at a range of concentrations) and 5 μCi/ml of [<sup>3</sup>H]dThd (50–80 Ci/mmol; New England Nuclear) were added. After a 3-h incubation at culture conditions, dishes were washed four times with ice-cold phosphate buffered saline followed by two washes with cold trichloroacetic acid (10% w/v). DNA was then extracted as described by Schmidt and Thannhauser (27) and quantitated by the method of Burton (28). [<sup>3</sup>H]dThd incorporation into DNA was determined by counting an amount of the extract solubilized with Aquassure in a Beckman LS 2800 liquid scintillation counter with a <sup>3</sup>H counting efficiency of 40–50%. The UDS activity in the respective cell lines was then expressed as the ratio of dpm/μg of DNA in drug treated cells to incorporation of [<sup>3</sup>H]dThd in DNA of untreated control cells.

## RESULTS

**Characteristics of Cisplatin Resistant Cell Lines.** Periodic IC<sub>50</sub> determinations have shown the degree of cisplatin resistance in the 2780<sup>CP8</sup> subline to be stable for at least 9 mo during subculture in drug-free medium (data not shown). The 2780<sup>CP8</sup> subline has a significantly shorter doubling time in monolayer culture and higher cloning efficiency (percentage of cells plated giving rise to colonies) in soft agarose than the parent line (Table 1). Both cell lines have a modal chromosome number of 46 as well as several common and distinctive clonal cytogenetic abnormalities (Table 1). Duplications of a specific portion of the q arm of chromosome 1 and the same breaking point at chromosome 6 are shared by both lines. Abnormalities noted only in the 2780<sup>CP8</sup> line include deletions of specific portions of the X chromosome and of chromosomes 6 and 20, an addition to the short arm of chromosome 13(13p+), and a translocation involving the q arms of chromosomes 4 and 7.

Table 1 Characteristics of the A2780 and 2780<sup>CP8</sup> human ovarian cancer cell lines

Characteristic	A2780	2780 <sup>CP8</sup>
Doubling time (h)	25.3 ± 1.0 <sup>a</sup>	22.1 ± 0.7 <sup>a, b</sup>
Cloning efficiency (%)	27.5 ± 2.9 <sup>a</sup>	36.4 ± 2.8 <sup>a, b</sup>
Relative cell volume <sup>c</sup>	1.00	1.08
Modal chromosome number	46	46
Chromosomal markers <sup>d</sup>	dup(1)(q23→q44) t(1;6)(q23;q21), 13pc <sup>e</sup>	del(X)(p21), dup(1) (q23→q24), del(6)(q21), t(4;7)(q32;q33), 13p+, del(20)(p21)

<sup>a</sup> Mean ± SE.

<sup>b</sup> *P* < 0.05 compared to value for the A2780 line.

<sup>c</sup> Determined with an electronic particle counter equipped with a channelizer.

<sup>d</sup> Nomenclature as per Paris Conference of 1971.

<sup>e</sup> pc, prominent centromere.

Homogeneously staining regions and double-minute chromosomes, karyotypic abnormalities associated with drug resistance acquired *in vitro* by other cell lines (29), were absent in the cisplatin-resistant cell line.

Cellular total GSH (GSH + GSH disulfide) content of the 2780<sup>CP8</sup> line is significantly higher than that of the A2780 line at all times during growth in monolayer culture (Fig. 1). After 4 days of culture the total GSH content (nmol/10<sup>6</sup> cells) was 10.7 ± 0.3 in 2780<sup>CP</sup> compared to 4.46 ± 0.4 in A2780. This difference in GSH content is not due to alterations in cell volume between these lines (Table 1). The greatest difference in total GSH content between these lines occurs at the earliest time after plating, day 2, but this is not a result of a differential response of the two lines to trypsinization or other aspects of subculture. To confirm that these marked early differences after plating were not due to differential trypsin effects between the cell lines, total GSH levels in single cell preparations similarly prepared but not plated were determined and found to be only minimally elevated at 3–6 h after trypsinization (1.3- and 1.2-fold in the A2780 and 2780<sup>CP8</sup> lines, respectively), and within 18–21 h returned to the base-line levels for cells in monolayer.

**Resistance and Cross-resistance Studies.** Cross-resistance to irradiation and other drugs commonly used in ovarian cancer therapy was assessed by concurrent comparisons of the sensitivity of a resistant subline to that of the parent line. By comparison of IC<sub>50</sub> drug doses with those of the parent line, the 2780<sup>CP8</sup> and 2780<sup>CP20</sup> sublines are 7.3 and 14-fold resistant to cisplatin, respectively (Table 2). Cross-resistance to irradiation and other drugs variably accompanies development of primary resistance *in vitro* in these lines (Tables 2 and 3). Only the 2780<sup>CP20</sup> subline is cross-resistant to Adriamycin, with a

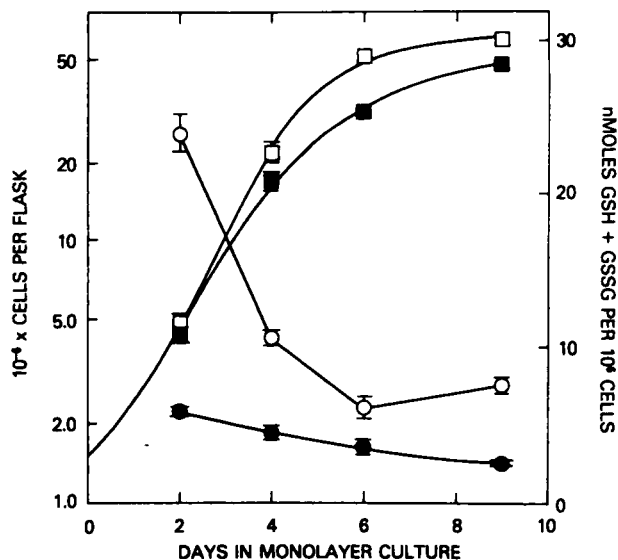


Fig. 1. Growth and total GSH levels of the A2780 and 2780<sup>CP8</sup> human ovarian cancer cell lines in monolayer culture. Cell numbers: ■, A2780; □, 2780<sup>CP8</sup>. Total GSH levels: ●, A2780; ○, 2780<sup>CP8</sup>. (Mean ± SE).

Table 2 Cross-resistance to melphalan or Adriamycin in cisplatin-resistant human ovarian cancer sublines

Subline	Cisplatin resistance <sup>a</sup>	Cross-resistance index <sup>b</sup> for:	
		Melphalan	Adriamycin
2780 <sup>CP8</sup>	7.3	2.1	1.1
2780 <sup>CP20</sup>	14.0	8.7	3.4
2780 <sup>CP70</sup>	39.0	11.0	ND <sup>c</sup>

<sup>a</sup> Ratio of IC<sub>50</sub> cisplatin dose in a resistant subline to that in the A2780 line.  
<sup>b</sup> Ratio of IC<sub>50</sub> drug dose in a resistant subline to that in the A2780 line.  
<sup>c</sup> ND, not determined.

Table 3 Comparative cytotoxicity of cisplatin and cisplatin analogues and cross-resistance to irradiation in the A2780 and 2780<sup>CP8</sup> human ovarian cancer cell lines

Drug	2780 <sup>CP8</sup>	A2780	Ratio <sup>d</sup>
Tetraplatin IC <sub>50</sub> μM <sup>b</sup>	0.96	0.41	2.3
Cisplatin IC <sub>50</sub> μM <sup>b</sup>	8.0	1.1	7.3
CHIP <sup>c</sup> IC <sub>50</sub> μM <sup>b</sup>	13	3.1	4.2
CBDCA IC <sub>50</sub> μM <sup>b</sup>	46	6.8	6.8
Irradiation	187 ± 19 <sup>d, e</sup>	85 ± 6 <sup>d</sup>	NA
D <sub>0</sub>	1.60 ± 0.18 <sup>d</sup>	2.14 ± 0.27 <sup>d</sup>	NA
Extrapolation number (N)			

<sup>a</sup> Degree of resistance (cisplatin) or cross-resistance (cisplatin analogues).  
<sup>b</sup> IC<sub>50</sub> doses from Fig. 3.  
<sup>c</sup> CHIP, iproplatin; CBDCA, carboplatin; NA, not applicable; D<sub>0</sub>, radiation dose reducing SF by a factor of 1/e.  
<sup>d</sup> Mean ± SE.  
<sup>e</sup> P < 0.001 compared to value for A2780 line.

CRI (ratio of IC<sub>50</sub> doses in a resistant and the parent cell line) of 3.4. Both cisplatin-resistant sublines are cross-resistant to melphalan, with the degree of cross-resistance paralleling that of primary cisplatin resistance. Cross-resistance to irradiation is also present at the lower level of primary cisplatin resistance. The D<sub>0</sub> (radiation dose reducing SF by a factor of 1/e) value of the 2780<sup>CP8</sup> subline (187 ± 19 rads) is significantly higher than that of the A2780 line (85 ± 6 rads). Thus, the irradiation dose required to reduce clonogenic cell surviving fraction by a factor of 1/e is more than 2-fold greater in the 2780<sup>CP8</sup> than in the parent line. The extrapolation numbers of these two lines also differ, but this does not reach statistical significance (P < 0.05).

**DNA Repair in A2780 and 2780<sup>CP8</sup>.** The cytotoxic lesions produced by melphalan and cisplatin are generally considered to be at the nuclear level through formation of DNA intra- and/or interstrand cross-links and/or DNA-protein cross-links (30). The effects of these DNA-reactive drugs on induction of UDS in A2780 and 2780<sup>CP</sup> as monitored by hydroxyurea refractory [<sup>3</sup>H]dThd incorporation (UDS assay) into DNA are shown in Fig. 2. As can be seen, melphalan (Fig. 2a) and cisplatin (Fig. 2b) treatment was associated with dose-dependent increases in UDS in the 2780<sup>CP</sup> cell line while the A2780 showed essentially no capacity to repair damage caused by these drugs.

**Relative Cytotoxicity of Cisplatin and 3-cisplatin Analogues.** Relative cytotoxicity and degree of cross-resistance with cisplatin were determined by concurrent comparisons in the A2780 and 2780<sup>CP8</sup> cell lines (Fig. 3 and Table 3). Only tetraplatin is more active, on a micromolar basis, than cisplatin. In both cell lines, the relative order of cytotoxicity was tetraplatin > cisplatin > iproplatin > carboplatin (most to least active). Development of primary resistance to cisplatin *in vitro* also results in variable cross-resistance to these 3-cisplatin analogues (Fig. 3 and Table 3). The degree of cross-resistance to carboplatin (CRI of 6.8) is comparable to the degree of primary resistance to cisplatin. In contrast, tetraplatin has minimal (CRI of 2.3) and iproplatin intermediate (CRI of 4.2) cross-resistance with cisplatin in the 2780<sup>CP8</sup> cell line. Cross-resistance to carboplatin increased (CRI of 16 in 2780<sup>CP20</sup>) as greater primary resistance to cisplatin was induced. However, even in the 2780<sup>CP70</sup> cell line which was approximately 40 times more resistant to cisplatin than A2780, the CRI remained less than 3 for tetraplatin.

DISCUSSION

The human cancer cell lines (2780<sup>CP8</sup>, 2780<sup>CP20</sup>, 2780<sup>CP70</sup>) with induced resistance to cisplatin have been shown to be useful in studying mechanisms of drug resistance and cross-resistance and in evaluating pharmacological means by which drug resistance can be decreased. Even though the stepwise selection procedure for cisplatin resistance *in vitro* may not be

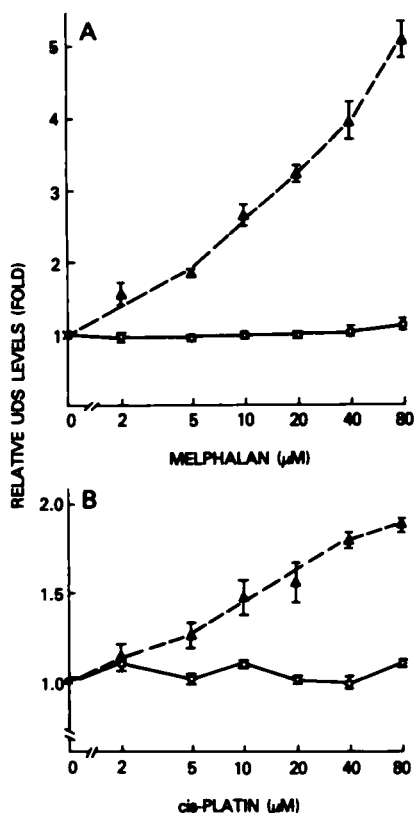


Fig. 2. Effect of melphalan (a) and cisplatin (b) on induction of UDS in human ovarian cancer cell lines A2780 (O) and 2780<sup>CP70</sup> ( $\Delta$ ). Values shown are ratios of hydroxyurea refractory [<sup>3</sup>H]dThd incorporation in the presence of increasing concentrations of drug to incorporation in the absence of drug; error bars, 1 SD. The base-line value (no treatment) for A2780 was  $67.2 \pm 4.4$  dpm/ $\mu\text{g}$  of DNA and for 2780<sup>CP70</sup> it was  $73.7 \pm 1.3$  dpm/ $\mu\text{g}$  of DNA.

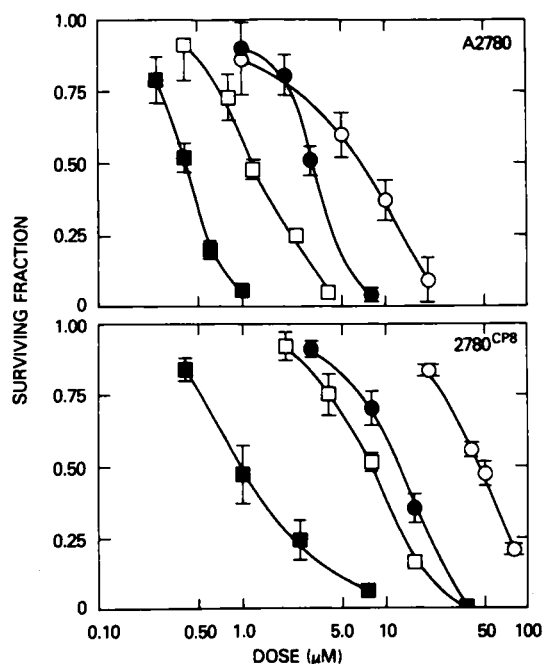


Fig. 3. Dose-response curves for cisplatin and cisplatin analogues in the A2780 (top) and 2780<sup>CP8</sup> (bottom) human ovarian cancer cell lines. ■, NSC-363812 D; □, cisplatin; ●, iproplatin (NSC-256927); and ○, carboplatin (NSC-241240) (mean  $\pm$  SE).

analogous to the mechanisms for clinical cisplatin resistance, the relative cytotoxicities and the nature of the dose-response relationships for cisplatin and its analogues in 2780<sup>CP8</sup> have proven useful in the design of "high dose" chemotherapy regi-

mens in ovarian cancer and in the preclinical evaluation of non-cross-resistant cisplatin analogues.

Compared to the sensitive cell line A2780, the cisplatin-resistant subline 2780<sup>CP8</sup> has a 2-fold increase in GSH content (31). Reduction of GSH by exposure of the cells to buthionine sulfoximine, a specific inhibitor of  $\gamma$ -glutamylcysteine synthetase, an enzyme required for GSH synthesis, decreases the resistance to cisplatin (31) and reverses the cross-resistance to melphalan (31) and irradiation (21). The exact mechanism by which GSH modulates cisplatin cytotoxicity is unknown. Among the possibilities under investigation in our laboratory are: (a) GSH may alter the transport of cisplatin; (b) GSH may inactivate cisplatin by forming an inactive GSH-cisplatin conjugate in the cytosol of resistant cells, although we have previously shown that there is no significant difference in the specific activities of GSH S-transferase, GSH peroxidase, or GSH reductase (31); (c) GSH may protect critical sites on DNA and decrease formation of the intrastrand *N*<sup>7</sup>-bidentate adduct on adjacent deoxyguanosines; (d) GSH may function in the repair of DNA damage.

The finding that induced resistance to cisplatin is accompanied by cross-resistance to other agents which cause DNA damage, *i.e.*, melphalan and irradiation, suggests that a common repair mechanism may be responsible at least in part for primary resistance to cisplatin and for the cross-resistance to melphalan and irradiation seen in 2780<sup>CP</sup>. Indirect evidence of a role for GSH in the repair of irradiation cytotoxicity has been provided by studies which have demonstrated that glutathione ester administered to lymphoid cells after irradiation was able to markedly decrease the lethality of the irradiation (32). To evaluate DNA repair as a possible mechanism of cisplatin resistance, we developed a variant of A2780 with marked resistance to the drug, 2780<sup>CP70</sup>, in the hope that supraclinical levels of resistance might increase the probability of detecting differences in overall repair activity using classical repair evaluation methodology with its limited sensitivity. Indeed, the preliminary screening experiments reported here indicated that cisplatin and melphalan dose-dependent increases in UDS are associated with induced resistance to cisplatin in 2780<sup>CP70</sup> while significant UDS is not observed in the parental cell line. Experiments using the more complex density shift methodology (33) and studies on the effect of repair inhibition on drug cytotoxicity are currently in progress. These studies should help clarify whether the UDS measured is indicative of DNA repair and if so the overall importance of DNA repair as a mechanism of anticancer drug resistance.

The dose-response curves for cisplatin and its analogues in the sensitive and resistant cell lines provided information of potential clinical relevance for the treatment of ovarian cancer. First, both the A2780 and 2780<sup>CP8</sup> cell lines have a steep dose-response curve with cisplatin exposure, Fig. 3, with the curve for the resistant subline simply displaced towards higher cisplatin concentrations. Thus, achievement of tumor cisplatin concentrations exceeding those obtained by conventional cisplatin administration may delay emergence of clinical drug resistance or produce responses in patients refractory to standard doses. Using hypertonic saline and vigorous chloruresis to circumvent the dose-limiting nephrotoxicity of cisplatin (9), we have demonstrated that high-dose cisplatin (200 mg/m<sup>2</sup>) produces a 32% response rate in ovarian cancer patients who were refractory to standard dose cisplatin regimens (8). In addition, it has also been demonstrated that higher drug levels associated with *i.p.* administration of cisplatin produce responses in patients who

have been refractory to the i.v. administration of standard cisplatin regimens (34).

The dose-response relationships of the platinum analogues may also be useful in identifying those analogues which may be particularly useful in the treatment of cisplatin-resistant tumors. Structure-activity relationships in drug-sensitive and -resistant murine tumors have demonstrated that resistance is a function of the ligands attached to the amine groups and not a function of the leaving groups (3, 10, 12). Thus, a high degree of cross-resistance would be expected between cisplatin and carboplatin inasmuch as the aquated intermediate of cisplatin (the active metabolite) is likely the same as the aquated form of carboplatin. In agreement with this was the finding that in the cisplatin-resistant cell line 2780<sup>CP8</sup>, there was marked cross-resistance with carboplatin (Table 3). It has recently been reported that patients refractory to full-dose therapy with cisplatin are unlikely to respond to therapy with carboplatin (35) thus providing support for the use of the cisplatin-resistant human ovarian cancer cell line 2780<sup>CP8</sup> to screen platinum analogues which may be of use in ovarian cancer patients. It is of particular interest that tetraplatin had the lowest degree of cross-resistance with the cisplatin-resistant cell line. Tetraplatin was evaluated in this system due to initial observations that it had activity against the subline of L1210 leukemia with acquired resistance to cisplatin. In contrast to other diamminocyclohexane platinum complexes, tetraplatin has a defined structure and sufficient purity, solubility, and stability for formulation development.<sup>5</sup> Tetraplatin is currently undergoing toxicity testing at the National Cancer Institute, and its marked activity in cisplatin-resistant human ovarian cancer cell lines suggests that this compound may be of use in those ovarian cancer patients who become resistant to cisplatin.

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<sup>5</sup> M. Wolpert-De Fillipes, National Cancer Institute, personal communication.