Mechanism-related Circumvention of Acquired cis-Diamminedichloroplatinum(II) Resistance Using Two Pairs of Human Ovarian Carcinoma Cell Lines by Ammine/Amine Platinum(IV) Dicarboxylates

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ABSTRACT

Acquired resistance to cisplatin has been generated in vitro in two human ovarian carcinoma cell lines: 41M, established from a previously untreated patient; and CH1, from a patient previously treated with cisplatin and cis-diammine-1,1-cyclobutane dicarboxylatoplatinum(II) (carboplatin). In neither cell line with acquired resistance did intracellular detoxification (via increased glutathione or metallothioneins) appear to be a major determinant of resistance. Resistance in 41McisR (resistance factor of 4.7) appeared to be due predominantly to a reduced platinum accumulation (levels were only 23.8% in 41McisR versus 41M). This was also reflected at the DNA level by a similar level of reduced DNA interstrand cross-links and total platinum-DNA adducts measured immediately after a 2-h exposure to cisplatin in 41McisR versus 41M. Conversely, for CH1cisR (resistance factor of 6.5), platinum accumulation, and initial numbers of DNA-interstrand cross-links and total DNA-platinum adducts were not significantly different from the parent CH1 line. This is suggestive of a resistance mechanism involving increased DNA repair or tolerance to platinum-DNA adducts operating in the CH1cisR/CH1 pair of lines. Cross-resistance to carboplatin and partial cross-resistance to the 1,2-diaminocyclohexane-containing agent, (trans-d,4)-1,2-diaminocyclohexane tetrachloroplatinum(IV) (tetraplatin), was observed in both pairs. However, two novel platinum(IV) ammine/amine dicarboxylates, ammine dibutyroldichlorocyclohexylamineplatinum(IV) (JM221) and ammine dibenzoatodichloropropylamineplatinum(IV) (JM244), completely circumvented resistance in 41McisR to produce some collateral sensitivity (resistance factors of 0.67 and 0.54, respectively) but showed cross-resistance in CH1cisR (resistance factors of 3.7 and 4.6). In contrast to the data for cisplatin, intracellular platinum levels were not significantly different between the 41M and 41McisR pair of cell lines after exposure to JM244. These results suggest that the ammine/amine platinum(IV) dicarboxylates, which show considerably greater in vitro cytotoxicity than cisplatin, are capable of circumventing acquired cisplatin resistance which is due to decreased intracellular accumulation but are not able to overcome resistance at the level of DNA platination and removal.

INTRODUCTION

The introduction of cisplatin-based chemotherapy into clinical oncology has made a major impact on the observed response rates of some tumor types, particularly testicular and ovarian carcinoma (1, 2). It was soon realized, however, that cisplatin possesses two notable limitations: its unfavorable toxicity profile (including neurotoxicity, nephrotoxicity, and severe nausea and vomiting) and a propensity for tumors to develop resistance to it. The severe side effects of cisplatin have been overcome largely by the development of the second-generation platinum drug, carboplatin (3, 4). However, while platinum-based chemotherapy is now able to offer patients a more acceptable level of morbidity, the results of both randomized cisplatin versus carboplatin and cross-over studies in ovarian cancer indicate that the two agents are effective against essentially the same population of tumors (5–8). Thus there remains an urgent need to discover new platinum drugs capable of circumventing cisplatin/carboplatin resistance.

There is considerable evidence suggesting that cisplatin exerts its cytotoxicity through binding to DNA to produce both intra- and interstrand cross-links (9). Laboratory-based investigations of platinum-induced resistance mechanisms have used both murine (e.g., P388, L1210, M5076) and human tumor models. Studies using human tumors have focused predominantly on tumor types such as ovarian carcinoma (10–11) and small cell lung cancer (12), where, typically, pairs of sensitive cell lines and variant cell lines with acquired cisplatin resistance have been established. Generally, these investigations allude to a multifocal basis for resistance involving one or more properties including accumulation, intracellular detoxification, chromatin binding, and DNA repair mechanisms (13–15).

Despite some 20 years of synthetic effort, there has been little progress to date in developing new platinum complexes capable of circumventing cisplatin/carboplatin resistance. Thus far the only complexes to reach clinical trials based on their preclinical circumvention of cisplatin resistance have evolved around the 1,2-diaminocyclohexane carrier ligand (16). Circumvention of resistance has generally been demonstrated using pairs of parent lines and murine leukemia (L1210 or P388) lines with acquired cisplatin resistance (17). The clinical relevance of such models has been questioned, however, by recent studies using other murine (18) and human (19) tumor models which have failed to reproduce the L1210 and P388 findings. 1,2-Diaminocyclohexane-containing (or closely related) complexes that have been evaluated clinically include 1,2-diaminocyclohexane-(4-carboxyphthalato)platinum(II) (JM82), TNO-6, and oxaliplatin; results have not been encouraging, primarily due to unacceptable toxicities (20–22). Another example of the 1,2-diaminocyclohexane-containing series of complexes, tetraplatin (Ormaplatin) (23), is currently in phase I clinical trials (24).

Our platinum-based drug discovery program is aimed at developing drugs capable of circumventing cisplatin/carboplatin resistance. With this objective in mind, we have established panels of in vitro (25) and in vivo (19) human ovarian carcinoma...
lines. These panels show a close in vitro versus in vivo correlation in cisplatin sensitivity/response (26). We have previously shown that a novel class of platinum(IV) complex, so-called ammine/amine dicarboxylates, possesses particularly encouraging in vitro cytotoxic properties (27–29). Some of these compounds are substantially more potent than cisplatin (up to 100-fold) and retain activity against cell lines possessing intrinsic resistance to cisplatin (29). A consistent feature of these novel complexes is the ammine/amine (or "mixed amine") carrier ligand; such complexes have been shown to exhibit greater cytotoxicity than corresponding diamine or bisamines (30).

In the present study, the two cell lines in our human ovarian carcinoma cell line panel showing the greatest sensitivity to cisplatin (41M and CH1) have been used to derive in vitro models of acquired cisplatin resistance. The mechanistic basis for resistance in both of these models has been determined. We have investigated the ability of two platinum(IV) ammine/amine dicarboxylates (JM221 and JM244) and tetraplatin to circumvent resistance in these paired cell lines.

MATERIALS AND METHODS

Cell Lines

Two human ovarian carcinoma cell lines, 41M and CH1, were used in this study. Their establishment and biological characterization have been described previously (25). 41M was derived from a previously untreated patient, while CH1 was established from a patient who had initially received only cisplatin (achieving a complete response) and subsequently carboplatin (achieving a partial response). Both cell lines were grown as monolayers in Dulbecco's modified Eagle's medium plus 10% fetal calf serum, 50 μg/ml gentamicin, 2.5 μg/ml amphotericin B, 2 mM l-glutamine, 10 μg/ml insulin, and 0.5 μg/ml hydrocortisone in a 10% CO₂, 90% air atmosphere. Cells were periodically checked and found to be free of Mycoplasma and were used in these studies from passage 25 to 50.

Anticancer Agents and Chemicals

The platinum drugs cisplatin, carboplatin, JM221, and JM244 were synthesized by and obtained from the Johnson Matthey Technology Centre (Reading, Berkshire, England). Tetraplatin was kindly provided by Dr. M. Wolpert-Defilippes (National Cancer Institute, Bethesda, MD). The structures of these agents are shown in Fig. 1.

Cadmium chloride, mitomycin C, vinblastine, chlorambucil, and sulforhodamine B were obtained from Sigma Chemicals (Poole, England). Methotrexate and 5-fluorouracil were obtained from David Bull laboratories (Warwick, England); the bleomycin was from Lundbeck, Ltd. (Luton, England); the Adriamycin was from Farmitalia Carlo Erba (Hertfordshire, England); and the melphalan was from Burroughs Wellcome (Bromley, England).

Assessment of Cytotoxicity

All agents were dissolved immediately before use in water, saline (for cisplatin and tetraplatin), absolute ethanol (for chlorambucil, JM221, and JM244), or 95% ethanol containing 2% hydrochloric acid (for melphalan). Where ethanol was used, the final concentration of solvent in the growth medium was 0.5%; this concentration had no inhibitory effect on cell growth.

Sulforhodamine B Assay

This was performed as described previously (26, 29, 31). Briefly, single viable cells were seeded into 96-well microtiter plates (5 × 10⁴ for CH1 and 1 × 10⁴ for 41M cells/well in 200 μl of growth medium). Agents were added to quadruplicate wells after overnight incubation. Exposure was for 96 h. Basic amino acid content per well was then analyzed using 0.4% sulforhodamine B in 1% acetic acid.

GSH Assay

Cellular GSH content was determined, in cells grown under conditions identical to those used for the assessment of cytotoxicity, as described previously (31). Approximately 1 × 10⁶ cells were seeded into 25-cm² flasks, and, after overnight incubation, cells were washed twice with ice-cold PBS. Cellular GSH was then extracted using 2 ml of ice-cold 0.6% sulfosalicylic acid followed by a 10-min incubation at 4°C with occasional shaking. Total GSH in the extract was then determined according to the method of Griffiths (33) and protein determined after solubilization in 2 ml of 1.0 N sodium hydroxide using the Lowry assay (32). The GSH content was expressed as nmol/mg protein.

Determination of Platinum Bound to DNA

Approximately 8 × 10⁶ cells were grown to near confluence in 175-cm² tissue culture flasks and harvested immediately after a 2-h cisplatin exposure. The DNA was extracted by a modification of the Kirby method (34) involving lysis (using 10 mM Tris, 10 mM EDTA, 0.15 M NaCl, and 0.4% sodium dodecyl sulfate) in the presence of 1 mg/ml proteinase K for 10 min at 60°C and then overnight at 37°C. DNA was then isolated following phenol extraction, ethanol precipitation, RNase treatment, reprecipitation and, finally, was hydrolyzed in 0.1 N sodium hydroxide using the Lowry assay (32). The GSH content was expressed as nmol/mg protein.

Alkaline Elution

DNA interstrand cross-linking was assessed by alkaline elution, essentially as described by Kohn et al. (36). The DNA of the cell lines was labeled by seeding 5 × 10⁵ cells in 25-cm² tissue culture flasks and
Growing for 24 h in the presence of 0.03 μCi/ml [14C]thymidine (specific activity, 51 mCi/mmol; Amersham International). A parallel flask of cells, to be used as an internal standard in the assay, was labeled overnight with 0.17 μCi/ml [methyl-3H]thymidine (specific activity, 5 Ci/mmol) plus 10^{-5} M unlabeled thymidine. Cells ([14C]-labeled) were exposed to varying concentrations of cisplatin (25, 50, and 100 μM) for 2 h while attached. In addition, an unexposed control flask was included in all experiments. Immediately after exposure, the drug was washed off using ice-cold PBS, and the cells were removed and added to ice-cold PBS. Test ([14C]-labeled) cells growing for 24 h in the presence of 0.03 nCi/ml [3H]thymidine (specific activity, 5 Ci/mmol) plus 10^{-5} M unlabeled thymidine were removed and added to ice-cold PBS. Test (l4C-labeled) cells were then gently added to duplicate 2-mm pore size 25-mm polycarbonate filters (Nucleopore Corporation) in 5 ml ice-cold PBS. Cells were then lysed by two additions of 10 ml lysis buffer containing 2% sodium dodecyl sulfate and 0.02 M disodium EDTA (pH 10). In the first 10 ml, proteinase K (0.5 mg/ml; Sigma Chemicals) was added immediately prior to use. DNA was then eluted at pH 12.2 using 10 ml of 0.1 M tetrapropylammonium hydroxide, containing 0.1% sodium dodecyl sulfate and 0.02 M EDTA. The elution rate was 0.011 ml/min (using a Watson Marlow peristaltic pump), and fractions were collected at 90-min intervals over 15 h. The [14C] and [3H] DNA radioactivity was then determined in each fraction and from the filters by liquid scintillation counting (Wallac 1410; Pharmacia). Results are expressed as fraction [14C] retained versus fraction [3H] (internal standard). In addition, DNA interstrand cross-link units/dalton x 10^6 were calculated using the following formula as described previously (37):

\[
\text{Crosslinks} = \left[ \left( \frac{1 - r}{1 - r_0} \right)^{1/2} - 1 \right] \times P_b
\]

where \( r \) and \( r_0 \) are the fractions of [14C]-labeled DNA for treated versus control cells remaining on the filter when 60% of [3H]-labeled DNA is retained on the filter, and \( P_b \) is the radiation-induced break probability/dalton.

In some experiments, unirradiated control experiments to test for the presence of cisplatin-induced single-strand breaks were also performed.

**Statistical Analysis**

Where appropriate, statistical significance was tested using a two-tailed Student's \( t \) test.

**RESULTS**

**Derivation of Acquired Cisplatin Resistance**

Resistance was generated in both 41M and CH1 using the same protocol. Cells were exposed to increasing concentrations of cisplatin (starting at approximately IC_{10}) over a 12- to 18-month period. Typically, cells were exposed at each concentration three times (lines being trypsinized as appropriate), after which the concentration was doubled. Exposure was continuous over 3 days; the drug was then removed, and the cells were exposed again when normal growth had resumed.

In both lines with acquired resistance, cell morphology was identical to that of the respective parent line under phase-contrast microscopy. In addition, population doubling times were unchanged (27 h for 41M and 17 h for CH1). Cisplatin concentration-effect curves for the two pairs of lines are shown in Fig. 2. Mean IC_{50} values were 0.26 for 41M and 1.23 for the counterpart with acquired resistance (resistance factor of 4.7) and 0.11 for CH1 and 0.71 for CH1cisR (resistance factor of 6.4). For 41M cisR, resistance appeared to be stable for at least 9 months in the absence of further maintenance doses of cisplatin. For CH1cisR, there was some indication of a partial loss of resistance over several months in the absence of drug. Therefore a maintenance dose of 0.5 μM/week was used; experiments were performed at least 2 weeks after drug removal. Using this protocol, resistance was stable.

**Mechanisms of Acquired Cisplatin Resistance**

Total intracellular platinum levels for 41M/cisR and CH1/cisR immediately after 2-h exposures to cisplatin are shown in Fig. 3. In the 41M pair of lines, platinum uptake was significantly lower (\( P < 0.05 \)) for the line with acquired resistance at each concentration tested. Across the six concentrations used (from 5 to 200 μM) platinum levels were an average of only 23.8% in 41M cisR compared to the parent line. In the CH1 pair of lines, there was no significant difference in uptake observed between the lines at any cisplatin concentration.

The results of experiments conducted to determine the possible role of increased intracellular detoxification of platinum through glutathione or metallothionein-derived mechanisms are shown in Table 1. The possible involvement of metallothioneins has been determined indirectly by measuring the sensitivity of the cell lines to cadmium chloride. While not a direct measure of metallothionein levels, other studies have shown a positive correlation between cellular sensitivity to cadmium chloride and metallothionein levels measured directly at the mRNA level (38). In neither pair did either of these intracellular detoxification mechanisms appear to contribute appreciably to the observed resistance.

The total amounts of platinum bound to DNA immediately after cisplatin exposure (10, 25, 50, and 100 μM × 2 h) are shown in Fig. 4. For the 41M pair of lines, total platinum-DNA adducts were significantly lower (\( P < 0.05 \)) in the line with acquired resistance at each cisplatin concentration. Across the four cisplatin concentrations used there was an average of 38.5% of the platinum adducts observed in the 41M cisR cell line compared to the parent line. In the CH1 pair of lines, no significant difference in the numbers of platinum-DNA adducts was observed between the lines at any cisplatin concentration.

Typical ISC profiles, as measured by alkaline elution immediately after a 2-h exposure of cells to varying concentrations of cisplatin (25, 50, and 100 μM) are shown in Fig. 5. For the 41M
cell line, increasing cisplatin concentrations resulted in a corresponding stepwise increase in numbers of ISC. However, for 41McisR, a shift from the control elution curve (indicative of ISC formation) was evident only at the highest cisplatin concentration of 100 μM. Cross-links per dalton × 10⁹ derived from the 41M and 41McisR elution curves showed that, across the three cisplatin concentrations, there was an average of only 21.3% ISC formed in 41McisR versus 41M (e.g., at 50 μM cisplatin ISC units/dalton × 10⁹ were 0.56 for 41M and 0.14 for 41McisR). For the two CH1 cell lines, similar ISC elution profiles were observed. There was no significant difference in ISC per dalton between CH1 and CH1cisR (e.g., at 50 μM cisplatin ISC units/dalton × 10⁹ were 0.51 for CH1 and 0.47 for CH1cisR). In control experiments performed using cisplatin (50 μM × 2 h) and unirradiated samples, the DNA eluted more slowly from untreated unirradiated cells, thus confirming as shown by others (e.g., Ref. 36) that no correction for single-strand break production was necessary.

Cross-Resistance Profiles

The ability of other platinum-based agents and other commonly used anticancer drugs to circumvent the acquired cisplatin resistance observed in these models has been evaluated. Table 2 shows cytotoxicity determinations (expressed in terms of IC₅₀ values) in both pairs of lines for cisplatin, carboplatin, tetraplatin, and the two novel platinum(IV) dicarboxylates JM221 and JM244. Contrasting cross-resistance profiles are apparent, especially for the dicarboxylates. This is shown, in terms of resistance factors, in Fig. 6. Cross-resistance (defined herein as a resistance factor of >1.5) to carboplatin and a partial cross-resistance to tetraplatin were observed for both pairs. Whereas JM221 and JM244 completely circumvented resistance in 41McisR, complete cross-resistance was observed for CH1cisR. However, the two dicarboxylates JM221 and JM244 appeared to produce some collateral sensitivity (resistance factor <1) to the 41McisR cell line. Furthermore, JM221 and JM244 exhibited considerably greater potency than cisplatin itself (in terms of IC₅₀ values, JM221 was 3.6-fold more potent than cisplatin to 41M; values were 25.6 in 41McisR, 16.7 in CH1, and 29.6 in CH1cisR). For JM244, values were 10 in 41M, 88 in 41McisR, 33 in CH1, and 47 in CH1cisR.

Table 3 shows IC₅₀ values and derived resistance factors for a series of 10 commonly used anticancer drugs. While all 10 circumvented cisplatin resistance in 41McisR, cross-resistance (resistance factor >1.5) was observed for melphalan, chlorambucil, mitomycin C, cytosine arabinoside, and etoposide in CH1cisR.

Accumulation of JM244 in the Pairs of Cell Lines

Total intracellular platinum levels for 41M/cisR and CH1/cisR immediately after 2-h exposures to JM244 (2.5 to 50 μM) are shown in Fig. 7. In contrast to the data for cisplatin, in both pairs of cell lines there was no significant difference in uptake between the paired cell lines across the concentration range investigated. For the 41M pair of lines, there was some indication at the highest two doses used (25 and 50 μM) of increased accumulation in the cell line with acquired cisplatin resistance. At equimolar doses, for all four cell lines, intracellular platinum levels were significantly higher after exposure to the dicarboxylate JM244 compared to cisplatin (P< 0.01). Across the concentration range 5 to 50 μM, intracellular platinum levels were a mean of 63-fold higher after exposure to JM244 versus cisplatin for the 41M cell line, 251-fold higher for the 41McisR line, 89-fold higher for the CH1 cells, and 150-fold higher for the CH1cisR line. A similar pattern of results was also obtained for the other dicarboxylate used in this study, JM221 (data not shown).

Table 1 GSH levels and sensitivity to cadmium chloride for the two pairs of cell lines

<table>
<thead>
<tr>
<th></th>
<th>41M*</th>
<th>41M cisR*</th>
<th>Fold difference</th>
<th>CH1*</th>
<th>CH1 cisR*</th>
<th>Fold difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH content (nmol/mg protein)</td>
<td>27.5 ± 4.9</td>
<td>28.7 ± 8.3</td>
<td>1.04</td>
<td>17.5 ± 2.5</td>
<td>21.6 ± 2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Sensitivity to cadmium chloride (IC₅₀ μM)</td>
<td>24.5 ± 6.5</td>
<td>28.1 ± 8</td>
<td>1.1</td>
<td>5.1 ± 2.2</td>
<td>2.4 ± 0.86</td>
<td>0.47</td>
</tr>
</tbody>
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* Mean ± SD; n = 3.
PLATINUM IV DICARBOXYLATES AND ACQUIRED CISPLATIN RESISTANCE

DISCUSSION

In this study, we have described two new cell line models of acquired cisplatin resistance in human ovarian carcinoma. This is a disease where successful clinical outcome is limited by the acquisition of platinum-based drug resistance. Previous studies using cell line models of acquired cisplatin resistance have alluded to a multifocal basis for resistance, with more than one mechanism often existing within an individual tumor cell line (see Refs. 13–15 for reviews). The resistance in the 41M and CH1 models used herein appears to have had an underlying biochemical basis involving contrasting mechanisms. In neither cell line with acquired resistance did intracellular detoxification (via increased glutathione or metallothioneins) appear to a major determinant of resistance. Indeed, for CH1cisR, some collateral sensitivity to cadmium chloride was apparent. However, it should be noted that, at least in some platinum-resistant tumor cell lines, increased levels of GSH (39, 40) or metallothionein (41) have been reported to play significant roles in determining resistance.

For the 41M pair of cell lines, these results suggest that a reduced platinum accumulation through the plasma membrane is likely to be a major determinant of acquired resistance. Intracellular platinum levels for the 41McisR cell line were 23.8% of those observed for 41M. This reduced uptake in 41McisR was reflected by similar degrees of reduced DNA platination...
and reduced DNA ISC compared with the parent 41M line. These uptake differences and parallel differences in numbers of DNA-DNA ISC are of a magnitude similar to those recently described in the PC-9 human non-small cell lung cancer parent and cisplatin-resistant lines (42). In addition, reduced platinum accumulation appears to play at least a partial role in the resistance of some of the various models of acquired cisplatin resistance, including both human (43–44) and murine tumor cell lines (45).

To date, the detailed biochemical mechanisms underlying the reduced uptake in any of these cell line models have not been fully elucidated. It remains unclear whether cisplatin enters cells by a passive diffusion process, is actively transported and reduced in the resistant cell lines and cisplatin-resistant lines (42). In addition, reduced platinum uptake differences and parallel differences in numbers of DNA-DNA ISC are of a magnitude similar to those recently described in the PC-9 human non-small cell lung cancer parent and cisplatin-resistant lines (42). In addition, reduced platinum accumulation appears to play at least a partial role in the resistance of some of the various models of acquired cisplatin resistance, including both human (43–44) and murine tumor cell lines (45).

For the CH1cisR line, uptake and levels of platinum reaching the DNA (both total adducts and ISC) were similar to those observed in the parent cells. This implies that resistance in CH1cisR may involve either an increased removal of platinum-DNA adducts or an increased tolerance to platinum-DNA adducts. Experiments to further investigate this (including measurements of the kinetics of platinum-DNA adduct removal) are in progress. As for reduced accumulation effects, a number of cisplatin-resistant cell lines have been shown to possess an enhanced capacity to remove platinum adducts from their DNA. These include the murine L1210 leukemia (49), the human A2780 ovarian carcinoma cell line (50), and a human testicular teratoma cell line with acquired cisplatin resistance (51).

Cross-resistance patterns have been determined for both platinum and other classes of anticancer drugs. In common with other such studies (10, 43, 51), both resistant lines investigated here exhibited a cross-resistance to carboplatin. Tetraplatin was developed for clinical trials largely because of its preclinical retention of activity against variants of the murine leukemia L1210 and P388 lines with acquired cisplatin resistance (17). However, tetraplatin failed to completely circumvent cisplatin resistance in either 41McisR or CH1cisR (resistance factors of 1.8 and 2.6, respectively).

In contrast, the two platinum(IV) ammine/amine dicarboxylates JM221 and JM244 exhibited a pattern of response distinctly different from that of both carboplatin and tetraplatin. While some collateral sensitivity was apparent with 41McisR, JM221 and JM244 failed to circumvent resistance in CH1cisR. These findings correlated directly with the level of platinum accumulation and DNA platination observed in the latter cell lines. Whereas a significant difference in intracellular platinum levels was observed between 41McisR and 41M after exposure to cisplatin, levels were not significantly different in this pair of cell lines after exposure to JM244. Furthermore, for equimolar doses, intracellular platinum levels were significantly higher after exposure to JM244 and JM221 compared to cisplatin for all four cell lines. These observations add support to our previous report that the striking in vitro potency advantage of JM221 over cisplatin is probably due to an enhanced uptake (29). These data also imply that the reduced uptake observed with 41McisR is not related to platinum complexes per se. However, the potential complexity of cisplatin cytotoxicity and the uncertainty involved in assigning individual cellular properties to resistance are reflected by the fact that although the CH1 parental cell line was more sensitive to cisplatin than was the parental 41M cell line, cisplatin accumulation and DNA platination were greater in the latter cell line. Nevertheless, the effectiveness of JM221 and JM244 against the 41M pair of cell lines signifies the existence of structure-activity requirements for the circumvention of acquired cisplatin resistance due to reduced uptake.

It is notable that, in CH1cisR, some cross-resistance was also observed to other cancer drugs (melphalan, chlorambucil, and mitomycin C) which produce bifunctional adducts on DNA.
This adds further indirect evidence that the mechanism of resistance in CH1cisR may involve an enhanced ability to cope with drug-induced DNA-DNA cross-links. It is interesting that Adriamycin, vinblastine, 5-fluorouracil, methotrexate, and bleomycin all retained activity against both cell lines with acquired cisplatin resistance, with bleomycin exhibiting some collateral sensitivity.

In summary, two new human ovarian carcinoma cell lines representing acquired cisplatin resistance have been established. Although the protocol for establishing the resistant lines was the same for both parent lines, resistance occurred through contrasting mechanisms. This suggests that, for each tumor cell type, the underlying mechanism of resistance to cisplatin may be via a predetermined phenotypic property. A novel class of platinum(IV) complex, ammine/amine dichlorodicarboxylates, while completely circumventing resistance in 41McisR (where resistance is due predominantly to reduced uptake), showed cross-resistance in CH1cisR (where resistance appears likely to have occurred at the DNA level). The circumvention of cisplatin resistance in 41McisR by the two dicarboxylates correlated with the intracellular accumulation data for these two agents where, in contrast to the marked difference in intracellular platinum levels observed between 41M and 41McisR after exposure to cisplatin, no significant difference in levels was observed after exposure to JM244 or JM221. These data could have implications for the discovery of novel platinum drugs capable of circumventing clinical resistance to cisplatin/carboplatin. It is conceivable that differing structural classes of platinum complex may be applicable to the circumvention of each of the three major known mechanisms of resistance (accumulation, detoxification, and DNA repair). To date, it is unclear which mechanism(s) may predominate at the clinical level. One recent study, however, has reported no correlation between metallothionein content and tumor sensitivity to platinum-based chemotherapy in ovarian cancer (52).

These cell line pairs provide useful models for mechanism-directed approaches to the evaluation of novel platinum-based complexes. We envisage using the lines in our ongoing platinum drug discovery program, together with previously described models of intrinsically cisplatin-resistant cell lines (25) and their in vivo xenografted counterparts.

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