Mechanisms of Acquired Resistance to the Orally Active Platinum-based Anticancer drug Bis-acetato-ammine-dichloro-cyclohexylamine Platinum (IV) (JM216) in Two Human Ovarian Carcinoma Cell Lines

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ABSTRACT

Acquired resistance to the p.o. active lipophilic platinum drug bis-acetato-ammine-dichloro-cyclohexylamine platinum (IV) (JM216) was generated in the 41M and CH1 human ovarian carcinoma cell lines, and their resistance mechanisms were compared to parallel cisplatin-resistant (cisR) cell lines. Intracellular platinum accumulation was not reduced in either 41M/JM216R or CH1/JM216R compared to the parent lines after JM216 exposure (1–100 μM for 2 h), and neither 41M/JM216R nor CH1/JM216R was cross-resistant to cadmium chloride, suggesting that metallothionein levels are not elevated. Resistance in 41M/JM216R (resistance factor, 1.9) appeared to be mainly due to elevated glutathione levels; levels were 1.6- and 1.8-fold higher in 41M/JM216R compared to 41M when expressed in terms of protein content and cell number respectively, reflected by a 1.7-fold reduction in total platinum bound to DNA in 41M/JM216R after JM216 exposure (10–100 μM for 2 h). This is in contrast to 41McisR, in which the major resistance mechanism was reduced intracellular accumulation. There was no difference between CH1/JM216R and CH1/JM216R in glutathione levels or levels of total platinum bound to DNA and DNA interstrand cross-links immediately after JM216 exposure (10–100 μM for 2 h or 25 μM for 2 h, respectively). In common with CH1cisR, increased DNA repair appeared to be the major resistance mechanism in CH1/JM216R (resistance factor, 6.2). Half times of removal of total platinum from DNA after JM216 exposure (25 μM for 2 h) were 20 h in CH1 and 11 h in CH1/JM216R; at 24 h after JM216 exposure (25 μM for 2 h), no removal of DNA interstrand cross-links was observed in CH1, while in CH1/JM216R 20% of cross-links had been removed. These results suggest that compared to cisplatin, acquired resistance to JM216 is less likely to occur through reduced accumulation. However, resistance can result from elevated glutathione levels or increased DNA repair, mechanisms also shown to be involved in cisplatin resistance.

INTRODUCTION

JM216 is a lipophilic ammine/ammine platinum (IV) dicarboxylate which has equivalent or superior in vitro cytotoxicity to cisplatin against panels of human cervical (1), small cell lung (2), and ovarian (3) carcinoma cell lines. In vivo JM216 is p.o. active, with antitumor selectivity against the murine ADJ/PC6 plasmacytoma after p.o. administration far superior to that observed with cisplatin and p.o. antitumor activity comparable to i.v. cisplatin against four human ovarian carcinoma xenografts (3). In mice, the dose-limiting toxicity with p.o. JM216 was myelosuppression; at the maximum tolerated dose, no hepatotoxicity was observed and gastrointestinal toxicity was lower than that observed with i.v. cisplatin (4). In contrast to cisplatin (i.v. or i.p.), p.o. JM216 caused no nephrotoxicity in mice or rats (5) and no neurotoxicity in rats (6) at the maximum tolerated dose. As a result of its antitumor activity and favorable toxicity profile, JM216 has undergone Phase I clinical trial as an p.o. administered drug (7, 8). The dose-limiting toxicity in patients was found to be myelosuppression, and JM216 is now undergoing Phase II clinical evaluation.

Although cisplatin is one of the most effective drugs available for the treatment of human testicular, ovarian, bladder, head and neck, small cell lung, and cervical cancer (9), its efficacy is limited by tumor cell resistance, present either at the onset of treatment (intrinsic) or after an initial response (acquired) (10). Studies using pairs of sensitive and acquired cisplatin-resistant murine and human tumor cell lines have shown that cisplatin resistance is multifocal, involving one or more of reduced intracellular accumulation, elevated intracellular thiol levels, and increased DNA repair (11). Our previous studies have shown that JM216 is able to circumvent acquired cisplatin resistance in both human ovarian (12) and cervical (1) carcinoma cell lines in which reduced accumulation plays a major role in resistance but not in cell lines in which resistance is due to increased DNA repair or elevated GSH levels (3).

The aims of this study were to determine whether the cisplatin resistance mechanisms described above are also involved in acquired resistance to JM216 in two human ovarian carcinoma cell lines, 41M established from a previously untreated patient, and CH1 established from a patient previously treated with cisplatin and carboplatin. Acquired cisplatin-resistant variants of these cell lines have been characterized previously; the major mechanisms of resistance in 41McisR and CH1cisR were reduced intracellular accumulation and increased DNA repair, respectively (13).

MATERIALS AND METHODS

Anticancer Drugs and Chemicals. Cisplatin, JM118, JM149, JM216, JM221, and JM335 were provided by the Johnson Matthey Technology Centre (Reading, UK), and tetraplatin was provided by Dr M. Wolpert-Defilippes (National Cancer Institute, Bethesda, MD). The structures of these drugs are shown in Fig. 1. 5-Fluorouracil was obtained from David Bull Laboratories (Warwick, UK), bleomycin from Lundbeck Ltd. (Luton, UK), Adriamycin from Pharmacia (Milton Keynes, UK), and melphalan from Burroughs Wellcome (Bromley, UK). Taxotere (RP56976, NSC628503, Docetaxel) was provided by Rhone-Poulenc Rorer (Antony, France). [14C]- and [3H]thymidine were obtained from Amersham International (Amersham, UK) and scintillation fluid from Canberra Packard (Pangbourne, UK). All other drugs and chemicals were obtained from Sigma Chemical Co. (Poole, UK).

Cell Lines. The human ovarian carcinoma cell lines 41M and CH1 were used in this study. Their establishment and biological characterization have been reported previously (14). Cell lines were grown as monolayers in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Imperial Laboratories, Andover, UK), 50 μM 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. Cells were used in these studies from passage 25–95.

Population-doubling Time Determinations. Cells (1 × 105) were seeded into 25-cm2 tissue culture flasks; cells in duplicate flasks were then detached by trypsinization and counted at 24-h intervals up to 120 h.

Assessment of Cytotoxicity. Cytotoxicity was assessed using the SRB assay as described previously (15). Between 5 × 103 and 1 × 105 cells were seeded

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: JM216, bis-acetato-ammine-dichloro-cyclohexylamine platinum (IV); GSH, glutathione; SRB, sulforhodamine B; IC(50), 50% inhibitory concentration; PBS, phosphate-buffered saline (pH 7.2); RF, resistance factor; Clmax, combination index at 50% cell kill.
GSH content was measured in cells grown under the same conditions as those used for the assessment of cytotoxicity as described previously (15). Cells (1 × 10^6) were seeded into triplicate 25-cm² tissue culture flasks and incubated overnight. GSH was then extracted into 2 ml 0.6% sulfo-soluble acetic acid and the GSH content of the extract determined by an enzymatic assay utilizing GSH reductase (20). The protein content of the extract was determined by Lowry assay (21) after solubilization in 2 ml of 1 M sodium hydroxide. Cellular GSH content was expressed as nmol GSH/mg protein and nmol GSH/10^6 cells, cell counts being carried out on parallel flasks.

Intracellular Platinum Accumulation. Approximately 3 × 10^6 cells growing exponentially in triplicate 25-cm² tissue culture flasks were exposed to JM216 or cisplatin (1–100 μM for 2 h). Cells were then washed with ice-cold PBS (3 × 25 ml), scraped and harvested in 0.5 ml PBS, and sonicated at 4°C (Soniprep 150, Fisons, Loughborough, UK). Platinum was determined by flameless atomic absorption spectrophotometry (Perkin Elmer Cetus Models 1100B and HGA700) and protein by Lowry assay (21) of a 50-μl aliquot digested in 200 μl of 1 M sodium hydroxide. Intracellular platinum levels were expressed as nmol platinum/mg protein.

Determination of Platinum Bound to DNA. Approximately 8 × 10^5 cells at near confluence in 175-cm² tissue culture flasks were exposed to JM216 (10–100 μM for 2 h). Cells were then harvested by trypsinization and the DNA extracted by a modification of the method of Kirby and Cook (22) as described previously (1). DNA was then hydrolyzed in 0.5 ml 0.2% nitric acid and the platinum content determined as above. DNA platination levels were expressed as nmol platinum/g DNA, the DNA content being determined from an aliquot by Burton assay (23).

Removal of Platinum from DNA. Approximately 8 × 10^5 cells at near confluence in 175-cm² tissue culture flasks [previously grown in the presence of 8 nCi/ml [14C]thymidine (specific activity, 53 mCi/mmol) for 48 h to label the DNA] were exposed to JM216 (25 μM for 2 h) and then incubated in drug-free medium for 0, 6, 12, 24, or 48 h before being harvested and DNA extracted as above. The nmol platinum/g DNA was determined at each time point; this was corrected for dilution by DNA synthesis during the postexposure period using the dilution factor (specific activity of DNA at time point/ specific activity of DNA at 0 h).

Removal of DNA Intercalation Cross-links. DNA-intercalation cross-links were measured by alkaline elution (24) as described previously (13). Test cells (8 × 10^5) were seeded into 25-cm² tissue culture flasks and the DNA labeled with [14C]thymidine. In addition, 2 × 10^5 internal standard cells (any cell line) were seeded into an 80-cm² tissue culture flask [previously grown in the presence of 5 nCi/ml [3H]thymidine. Test cells were then exposed to JM216 (25 μM for 2 h) and then incubated in drug-free medium for 0, 5, 15, or 24 h. An unexposed control flask was also included in all experiments. Test and normal standard cells were harvested by trypsinization, resuspended in PBS, and irradiated with 5 and 2 Gy, respectively. Cells (2 × 10^6) of a 1:1 mix of test and internal standard cells were added to polycarbonate filters; the cells were lysed and the DNA eluted. The [14C] and [3H] DNA radioactivity was determined in each fraction and the filters by liquid scintillation counting. The interstrand cross-linking index was calculated using the formula:

\[
\text{Interstrand cross-linking index} = \left( \frac{1 - R_0}{1 - R_i} \right)^{1/2} - 1
\]

where R₀ and Rᵢ are the fractions of 14C-labeled DNA retained on the filter for control and treated cells when 30% of 3H-labeled DNA is retained on the filter.

Statistical Analysis. Statistical significance was tested using the Student's t test.

RESULTS

Development of Acquired JM216 Resistance in 41M and CH1

Resistance to JM216 was generated in 41M and CH1 using the protocol used previously for the generation of cisplatin resistance in these cell lines (13). Cells were exposed to increasing concentrations of JM216 over a 17-month period (starting concentration, 20 nM; final concentration, 1 μM and 1.5 μM for 41M and CH1, respectively).
Cells were exposed to each concentration a minimum of 3 times, after which the concentration was doubled. Exposure was continuous over 3 days; the drug was then removed, and the cells were exposed again when they had regained their normal growth properties (after approximately 1 week). Table 1 shows the cytotoxicity of JM216 in 41M and CH1 and the derived cell lines, 41M/JM216R and CH1/JM216R, using a 96-h or 2-h exposure SRB assay. 41M/JM216R was 1.9-fold resistant to JM216 after 96-h exposure and 2.7-fold resistant after 2-h exposure. Further exposure of 41M/JM216R to increasing concentrations of JM216 (up to 2 μM) over an 8-month period did not increase the resistance of this cell line. CH1/JM216R was 6.2-fold resistant to JM216 after 96-h exposure and 4.9-fold resistant after 2-h exposure. The resistance of both 41M/JM216R and CH1/JM216R was stable in the absence of maintenance doses of JM216 for at least 6 months. The cell morphology of the two JM216-resistant lines was identical to that of the respective parent lines under phase contrast microscopy, and no difference in population-doubling time was observed (27 h for 41M pair and 17 h for CH1 pair).

Mechanisms of Acquired JM216 Resistance in 41M/JM216R and CH1/JM216R

Intracellular Platinum Accumulation. Fig. 2 shows intracellular platinum accumulation in the 41M pair (Fig. 2A) and CH1 pair (Fig. 2B) of cell lines immediately after 2-h exposure to JM216 at a range of concentrations from 1–100 μM. Intracellular platinum levels were not reduced in either 41M/JM216R or CH1/JM216R compared to their respective parent lines at any of the 6 concentrations used.

Intracellular Thiol Levels. GSH levels in the two pairs of cell lines are shown in Fig. 3. GSH levels were elevated in 41M/JM216R compared to 41M by 1.6-fold (P = 0.02) when expressed in terms of protein content and 1.8-fold (P = 0.07) when expressed in terms of cell number. There was no significant difference in GSH levels between CH1/JM216R and CH1 when expressed in terms of protein content (P = 0.55) or cell number (P = 0.18). The sensitivity of the two pairs of cell lines to cadmium chloride was measured using a 96-h exposure SRB assay. IC50 values (μM, mean ± SD, n ≥ 3) were 16.7 ± 1.3 in 41M, 15.9 ± 1.7 in 41M/JM216R (RF, 0.95), 5.5 ± 1.2 in CH1, and 2.5 ± 0.9 in CH1/JM216R (RF, 0.45). Therefore, neither 41M/JM216R nor CH1/JM216R was cross-resistant to cadmium chloride, suggesting that metallothionein levels are not elevated compared to the parent cell lines.

Platinum-DNA Binding. Levels of total platinum bound to DNA immediately after exposure to JM216 (10–100 μM for 2 h) are shown in Fig. 4, A (41M pair) and B (CH1 pair). Platinum-DNA binding was significantly reduced (P = 0.03) in 41M/JM216R compared to 41M by 1.6-fold (P = 0.02) when expressed in terms of cell number. There was no significant difference in GSH levels between CH1/JM216R and CH1 when expressed in terms of protein content (P = 0.55) or cell number (P = 0.18). The sensitivity of the two pairs of cell lines to cadmium chloride was measured using a 96-h exposure SRB assay. IC50 values (μM, mean ± SD, n ≥ 3) were 16.7 ± 1.3 in 41M, 15.9 ± 1.7 in 41M/JM216R (RF, 0.95), 5.5 ± 1.2 in CH1, and 2.5 ± 0.9 in CH1/JM216R (RF, 0.45). Therefore, neither 41M/JM216R nor CH1/JM216R was cross-resistant to cadmium chloride, suggesting that metallothionein levels are not elevated compared to the parent cell lines.

DNA Repair. Fig. 5A shows the removal of total platinum from DNA immediately after exposure to JM216 (10–100 μM for 2 h) are shown in Fig. 4, A (41M pair) and B (CH1 pair). Platinum-DNA binding was significantly reduced (P = 0.03) in 41M/JM216R compared to 41M by an average of 1.7-±0.2 (SD)-fold across the 4 concentrations. In CH1/JM216R, platinum-DNA binding was not reduced compared to CH1 at any of the 4 concentrations.

DNA Repair. Fig. 5A shows the removal of total platinum from DNA immediately after exposure to JM216 (10–100 μM for 2 h) are shown in Fig. 4, A (41M pair) and B (CH1 pair). Platinum-DNA binding was significantly reduced (P = 0.03) in 41M/JM216R compared to 41M by an average of 1.7-±0.2 (SD)-fold across the 4 concentrations. In CH1/JM216R, platinum-DNA binding was not reduced compared to CH1 at any of the 4 concentrations.

Table 1 Resistance of 41M/JM216R and CH1/JM216R to JM216 (96- or 2-h exposure SRB assay)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>JM216 96-h IC50 (μM)a</th>
<th>RFb</th>
<th>JM216 2-h IC50 (μM)a</th>
<th>RFb</th>
</tr>
</thead>
<tbody>
<tr>
<td>41M</td>
<td>0.60 ± 0.12</td>
<td>1.0</td>
<td>6.0 ± 1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>41M/JM216R</td>
<td>1.16 ± 0.38</td>
<td>1.9</td>
<td>18.3 ± 6.0</td>
<td>2.7</td>
</tr>
<tr>
<td>CH1</td>
<td>0.07 ± 0.010</td>
<td>1.9</td>
<td>2.1 ± 0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>CH1/JM216R</td>
<td>0.45 ± 0.09</td>
<td>6.2</td>
<td>10.3 ± 2.6</td>
<td>4.9</td>
</tr>
</tbody>
</table>

aMean ± SD (n ≥ 3 experiments).
b RF = IC50 of the JM216-resistant cell line/IC50 of the parent cell line.
cross-link levels were 1.5-fold lower in CH1/JM216R compared to CH1.

Table 2 shows the interaction between JM216 and the DNA polymerase α and δ inhibitor aphidicolin in CH1 and CH1/JM216R cells using the SRB assay. CH1/JM216R was not cross-resistant to aphidicolin (RF, 1.3). The CI₅₀ was determined by median effect analysis after 96-h exposure to both drugs and 2-h exposure to both drugs followed by 94-h exposure to aphidicolin. A CI₅₀ of 1 indicates an additive interaction, a CI₅₀ of less than 1 indicates synergism, and a CI₅₀ of greater than 1 indicates antagonism (16). In both CH1 and CH1/JM216R cells, a CI₅₀ of greater than 1 was observed after both 96- and 2-h exposure to JM216/aphidicolin, indicating that the interaction between JM216 and aphidicolin was antagonistic.

Cross-resistance of 41M/JM216R and CH1/JM216R

Platinum-based Anticancer Drugs. Fig. 6 shows the cross-resistance profile of the two JM216-resistant cell lines to other platinum-based anticancer drugs using a 96-h exposure SRB assay. 41M/JM216R was 1.9-fold resistant to JM216, and a similar level of resistance was observed to tetraplatin, JM118, JM149, JM221, and JM335. A higher level of resistance was observed to cisplatin (RF, 4.3). Fig. 7 shows intracellular platinum accumulation in 41M and 41M/JM216R immediately after 2-h exposure to cisplatin at a range of concentrations from 1–100 μM; intracellular platinum levels were 1.3- ± 0.1-fold lower (P < 0.01) in 41M/JM216R compared to 41M (mean ± SD across the entire concentration range). CH1/JM216R was 6.2-fold resistant to JM216 and cross-resistant to cisplatin, tetraplatin, JM118, JM149, and JM221 (RF > 3). Only 1.8-fold resistance was observed to the novel trans-platinum complex JM335.

Nonplatinum Anticancer Drugs. The cross-resistance profile (96-h exposure SRB assay) of 41M/JM216R and CH1/JM216R to nonplatinum anticancer drugs is shown in Table 3. 41M/JM216R (JM216 RF = 1.9) showed complete cross-resistance to melphalan, chlorambucil, mitomycin C, and bleomycin; a low level of resistance to etoposide; and collateral sensitivity to Adriamycin, vinblastine, 5-fluorouracil and taxotere. CH1/JM216R (JM216 RF = 6.2) was cross-resistant to melphalan, chlorambucil, and mitomycin C. A low level of resistance was observed to vinblastine, 5-fluorouracil, and taxotere, while Adriamycin and etoposide can be considered to completely circumvent resistance (RF < 1.5). Collateral sensitivity was observed to bleomycin.

DISCUSSION

The aims of this study were to determine the mechanisms underlying acquired resistance to the novel p.o. active platinum-based anticancer drug JM216 in two human ovarian carcinoma cell lines, 41M and CH1. Cisplatin exerts its cytotoxicity by the formation of intra- and interstrand cross-links on DNA (25); JM216 also forms both intra- and interstrand DNA cross-links. Acquired resistance to cisplatin can be mediated by factors which reduce platinum-DNA adduct formation, i.e., reduced intracellular accumulation/increased inactivation by intracellular thiols, or by increased repair of platinum-DNA adducts (11). These properties were investigated in 41M/
Table 2 Interaction of aphidicolin with JM216 in CIII and CH1/JM216R (SRB assay)

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>Cell line</th>
<th>Ratio JM216: aphidicolin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>JM216 IC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Apophidicolin IC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>JM216/Aphidicolin IC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 h JM216/96 h aphidicolin</td>
<td>CH1</td>
<td>1:1</td>
<td>0.066 ± 0.005</td>
<td>0.14 ± 0.04</td>
<td>0.12 ± 0.02</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>CH1/JM216R</td>
<td>4:1</td>
<td>0.47 ± 0.08</td>
<td>0.18 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>2 h JM216/96 h aphidicolin</td>
<td>CH1</td>
<td>30:1</td>
<td>2.1 ± 0.7</td>
<td>0.14 ± 0.04</td>
<td>1.4 ± 0.5</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>CH1/JM216R</td>
<td>100:1</td>
<td>10.3 ± 2.6</td>
<td>0.18 ± 0.02</td>
<td>8.2 ± 1.2</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio determined from the IC<sub>50</sub> ratio in a preliminary experiment.

<sup>b</sup> Mean ± SD (n = 3 experiments).

ACQUIRED RESISTANCE TO JM216 IN OVARIAN CARCINOMA

Reduced intracellular platinum accumulation is the major mechanism of resistance in 41McisR, resulting in similarly reduced levels of platinum-DNA adducts, while no reduction in intracellular platinum accumulation was observed in CH1cisR. It has been postulated that cisplatin accumulation is partly due to passive diffusion and partly to facilitated diffusion through a gated channel, and that reduced cisplatin accumulation in resistant cells may result from inactivation of the channel protein (29). The accumulation of JM216, which is more lipophilic than cisplatin and presumably less dependent on the gated channel for entry into the cell, was not reduced in 41McisR, and as a result JM216 circumvented resistance in this cell line (12). Intracellular platinum accumulation was not reduced in either 41M/JM216R or CH1/JM216R compared to the respective parent lines after JM216 exposure. This is consistent with the hypothesis that the gated channel is not required for JM216 accumulation; therefore, acquired resistance to JM216 in 41M, in contrast to cisplatin, cannot be mediated through reduced accumulation. This may account for the difficulty observed in developing JM216 resistance in this cell line; 41M/JM216R was only 1.9-fold resistant to JM216 after more than 2 years of exposure.

Overexpression of the major intracellular protein thiol, metallothionein, has been observed in a number of acquired cisplatin-resistant human tumor cell lines (30). However, neither 41M/JM216R nor CH1/JM216R was cross-resistant to cadmium chloride, suggesting that metallothionein levels are not elevated compared to the respective parent cell lines. In fact, collateral sensitivity to cadmium chloride was observed in CH1/JM216R (RF, 0.45). Similar results were obtained previously for 41McisR and CH1cisR. Elevated levels of GSH,
the major intracellular nonprotein thiol, have been observed in acquired cisplatin-resistant human ovarian (31), cervical (1), colon (32), and small cell lung (33) carcinoma cell lines, although GSH levels were not elevated in 41McisR or CH1cisR. In contrast, GSH levels were significantly elevated in 41M/JM216R compared to 41M, reflected by a 1.7-fold reduction in total platinum-DNA binding in 41M/JM216R immediately after JM216 exposure. Thus, JM216 resistance in JM216R appears to be due mainly to an elevation in GSH levels, although other resistance mechanisms such as DNA repair may also contribute. No difference was observed in GSH levels between CH1/JM216R and CH1, reflected by no difference in the levels of total platinum bound to DNA or DNA interstrand cross-links immediately after JM216 exposure.

In CH1cisR there was no reduction in initial levels of total platinum bound to DNA or DNA interstrand cross-links after cisplatin exposure, suggesting that the major mechanism of resistance in both CH1cisR and CH1/JM216R is increased repair of platinum-DNA adducts. Increased DNA repair has been observed in acquired cisplatin-resistant variants of human ovarian carcinoma (34, 35), cervical carcinoma (36, 37), and testicular teratoma (38) cell lines. Removal of both total platinum and interstrand cross-links from DNA after JM216 exposure was enhanced in CH1/JM216R; the half time of removal of total platinum from DNA was 20 h in CH1 and 11 h in CH1/JM216R. In CH1, no removal of DNA interstrand cross-links was observed up to 24 h after exposure, whereas 20% of interstrand cross-links had been removed at this time point in CH1/JM216R. Therefore, it appears that increased genomic DNA repair is involved in JM216 resistance in CH1/JM216R.

Repair of cisplatin-DNA adducts occurs via nucleotide excision repair (39), a process which requires DNA polymerase activity. DNA polymerases α and β are overexpressed in acquired cisplatin-resistant variants of human HCT8 colon and A2780 ovarian (40, 41), but not 2008 ovarian (42), carcinoma cell lines. CH1/JM216R was not cross-resistant to aphidicolin, a specific inhibitor of DNA polymerases α (43) and δ (44), suggesting that DNA polymerases α and δ are not overexpressed compared to CH1. Aphidicolin has been shown to inhibit DNA repair after cisplatin damage and enhance cisplatin cytotoxicity in acquired cisplatin-resistant human A2780 ovarian (34, 35) and HeLa cervical carcinoma (36, 37) cell lines, in which increased DNA repair is responsible for resistance. Using median effect analysis, the interaction between aphidicolin and cisplatin was shown to be synergistic in acquired cisplatin-resistant, but not parent, human 2008 ovarian carcinoma cells under conditions of short-term exposure to drug combinations, while synergism was not observed in either cell line when exposure to drug combinations was continuous (42). In contrast, the interaction between aphidicolin and JM216 was antagonistic in both CH1 and CH1/JM216R under conditions of 2- or 96-h exposure to drug combinations, suggesting that DNA polymerases α and δ are not important determinants of JM216 cytotoxicity in these cell lines.

41M/JM216R was cross-resistant to melphalan, chlorambucil, mitomycin C, and bleomycin; elevated GSH levels have been shown previously to protect against the cytotoxic effects of these drugs (45–48). 41M/JM216R showed a general cross-resistance to other platinum-based anticancer drugs. In particular, a high level of resistance was observed to cisplatin (RF, 4.3), associated with a 1.3-fold reduction in intracellular platinum accumulation in 41M/JM216R compared to 41M after cisplatin exposure. CH1/JM216R, like CH1cisR, was cross-resistant to melphalan, chlorambucil, and mitomycin C, drugs which also exert their cytotoxicity by the formation of bifunctional adducts on DNA; acquired cisplatin-resistant human testicular teratoma (38) and cervical carcinoma (37) cell lines, in which resistance is due to increased DNA repair, are also cross-resistant to these drugs. CH1/JM216R showed collateral sensitivity to bleomycin (RF, 0.4), a property which has been observed previously in an acquired cisplatin-resistant variant of the A2780 human ovarian carcinoma cell line (49). CH1/JM216R was cross-resistant to other cis-platinum complexes including JM149. JM335, the trans-isomer of JM149, which has been shown to form DNA single strand breaks but no DNA inter- or intrastrand cross-links in CH1 (50), showed only a low level of cross-resistance (RF, 1.8).

In summary, reduced intracellular drug accumulation is a common feature of acquired resistance to cisplatin, a phenomenon which represents a major clinical problem. JM216 is a novel p.o. active platinum-based anticancer drug in clinical trial which has been shown previously to circumvent this resistance mechanism in both human ovarian (12) and cervical (1) carcinoma cell lines. In addition, this study suggests that acquired resistance to JM216 cannot be mediated through reduced drug accumulation; resistance is caused by increased

<table>
<thead>
<tr>
<th>Drug</th>
<th>41M 96-h IC50 (µM)×10^-3</th>
<th>41M/JM216R 96-h IC50 (µM)×10^-3</th>
<th>RF×10^-3</th>
<th>CH1 96-h IC50 (µM)×10^-3</th>
<th>CH1/JM216R 96-h IC50 (µM)×10^-3</th>
<th>RF×10^-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan</td>
<td>4.0 ± 0.5</td>
<td>9.7 ± 0.4</td>
<td>2.4</td>
<td>1.4 ± 0.4</td>
<td>5.8 ± 0.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>14.0 ± 1.0</td>
<td>32.5 ± 6.5</td>
<td>2.3</td>
<td>5.9 ± 0.9</td>
<td>22.3 ± 6.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>0.074 ± 0.006</td>
<td>0.054 ± 0.002</td>
<td>0.7</td>
<td>0.0041 ± 0.0002</td>
<td>0.0056 ± 0.0010</td>
<td>1.4</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>0.16 ± 0.02</td>
<td>0.42 ± 0.07</td>
<td>2.6</td>
<td>0.032 ± 0.008</td>
<td>0.15 ± 0.01</td>
<td>4.8</td>
</tr>
<tr>
<td>Etoposide</td>
<td>0.92 ± 0.06</td>
<td>1.3 ± 0.2</td>
<td>1.4</td>
<td>0.12 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>1.2</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>0.0016 ± 0.0006</td>
<td>0.00082 ± 0.00024</td>
<td>0.5</td>
<td>0.00066 ± 0.00011</td>
<td>0.0011 ± 0.0003</td>
<td>1.7</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>1.9 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>1.8</td>
<td>0.32 ± 0.03</td>
<td>0.12 ± 0.01</td>
<td>0.4</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>43.3 ± 5.8</td>
<td>21.5 ± 2.5</td>
<td>0.5</td>
<td>2.2 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Taxotere</td>
<td>0.0036 ± 0.0013</td>
<td>0.0026 ± 0.0006</td>
<td>0.7</td>
<td>0.0015 ± 0.0002</td>
<td>0.0025 ± 0.0005</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*a* Mean ± SD (n = 2 experiments).

*b* RF = IC50 of the JM216-resistant cell line/IC50 of the parent cell line.

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in intracellular GSH levels or increased DNA repair, mechanisms which have also been implicated in cisplatin resistance.

REFERENCES


Mechanisms of Acquired Resistance to the Orally Active Platinum-based Anticancer drug Bis-acetato-ammine-dichloro-cyclohexylamine Platinum (IV) (JM216) in Two Human Ovarian Carcinoma Cell Lines

Kirste J. Mellish and Lloyd R. Kelland