Anomalous Relationship between Cisplatin Sensitivity and the Formation and Removal of Platinum-DNA Adducts in Two Human Ovarian Carcinoma Cell Lines in Vitro

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ABSTRACT

Two human ovarian tumor cell lines (SK-OV-3 and TR175), established from patients previously treated with alkylating agents, but not with cisplatin, expressed >23-fold differences in cisplatin sensitivities in vitro. Cisplatin resistance in SK-OV-3 cells appeared to be associated with increased levels of glutathione and activities of glutathione reductase and glutathione peroxidase, with reduced catalase activity. No significant modification of drug uptake was noted and there was only marginally lower (16%) total platination of DNA, measured immunologically, in these cells compared with the more sensitive TR175 cell line. SK-OV-3 cells, however, showed a significantly lower overall ability to remove drug-induced DNA damage, with an apparent inability to remove either the major DNA-DNA intrastand cross-links in the sequence pGpG or the adducts cis-Pt(NH3)2(dGMP)2, although by alkaline elution repair of DNA-DNA interstrand cross-links was demonstrated. Significantly more of these interstrand cross-links were induced in these resistant cells. These data provide evidence for the involvement of altered glutathione metabolism and increased tolerance of certain types of drug-induced DNA damage as factors associated with the resistance phenotype of SK-OV-3 cells. Paradoxically, however, although the highly cisplatin-sensitive TR175 cells had lower glutathione levels this was not reflected in significantly higher total platination of DNA, and these cells appeared to be proficient in removing all the major platinum-DNA adducts quantitated in this study. Mechanisms responsible for this relative sensitivity to cisplatin remain to be identified.

INTRODUCTION

Cisplatin, a clinically important antitumor agent with activity against a wide spectrum of human tumors (1, 2), has proved particularly useful in treating ovarian cancer. In patients with advanced disease cisplatin-based combination chemotherapy has resulted in increased complete response rates, with some studies reporting prolonged overall survival compared to single agent therapy (1, 3, 4). However, the majority of patients with bulky disease are not cured, and the emergence of resistance is a common consequence of therapy (3–6). Mechanisms underlying resistance to cisplatin are clearly multifactorial. The following factors have been implicated, although a causal relationship may not have been established: reduced drug accumulation (7, 8); enhanced inactivation of the drug frequently involving thiol compounds (9, 10); differential DNA damage; and apparent tolerance of that damage and/or its repair (11–15). Most of this evidence has been derived from experimental model systems involving rodent or human tumor cell lines in which cisplatin resistance has been selected for, generally under in vitro or, less commonly, in vivo conditions. Hamilton et al. (9) and Schilder et al. (10), however, have also examined these various parameters in human ovarian tumor cell lines established from either previously untreated or previously treated patients. In our earlier studies (16), in which we described the establishment of three new human ovarian tumor cell lines in vitro, we reported that when compared with the SK-OV-3 human ovarian tumor line originally described by Fogh et al. in 1977 (17) a wide range (>23-fold) of cisplatin sensitivities was expressed. Specifically the SK-OV-3 cell line appeared to be resistant to cisplatin and, indeed, to other antitumor agents including Adriamycin, while line TR175 appeared to be hypersensitive to the cytotoxic effects of cisplatin. In the present study, therefore, our objective was to characterize the various parameters associated with differential cisplatin sensitivities in these two ovarian human tumor cell lines, both established from patients not previously treated with cisplatin, so as to determine whether the mechanisms operating in the more resistant subline were similar to or different from those associated with cisplatin resistance in in vitro drug-selected sublines.

MATERIALS AND METHODS

Cell Lines. SK-OV-3 cells, originally described by Fogh et al. (17) as derived from malignant ascites of a patient treated by thiopeta, were obtained from the American Type Culture Collection (Rockville, MD). They have been maintained in culture with weekly passaging (1:5) in Dulbecco's modification of Eagle's medium plus 10% fetal calf serum (Gibco Paisley, Scotland). Line TR175 was initiated from malignant ascites from a patient presenting with a serious stage III cystadenocarcinoma of the ovary. Primary treatment was surgery with i.p. installation of cyclophosphamide, followed by chlorambucil p.o. Full details of the derivation of this line have been provided earlier (16). Ham's F-12 medium (Gibco) plus 10% fetal calf serum were used to maintain these cells in an atmosphere of 5% CO2 in air at 37°C. Details of the growth characteristics of these two cell lines have been published previously (16).

Drugs and Chemicals. Sources of the drugs and chemicals used were as follows. Cisplatin, cadmium chloride, and BSO1 were purchased from Sigma Chemicals (Poole, England); carboplatin (CBDCA; NSC-2412240–2) was kindly provided initially by Dr. A. H. Calvert (Institute of Cancer Research, Sutton, Surrey, England) and subsequently by the Bristol-Myers Company, Inc. (Evansville, IN); Adriamycin was a gift from Farmitalia Carlo Erba (Milan, Italy); vincristine was donated by Lederle Laboratories (Gosport, Hampshire, England); etoposide was provided by the Bristol-Myers Company.

Cytotoxicity Assays. Cytotoxicity assays were carried out using the soft agar clonogenic assay method of Courtenay et al. (18) with modifications described previously (16). While SK-OV-3 cells cloned readily in soft agar with a 5–10% efficiency, this was not the case for TR175 cells which had only an approximately 0.5% cloning efficiency. All assays were performed in triplicate on a minimum of two independent occasions. Concentrations of drug reducing colony formation to 50% of control levels were estimated from full dose-response curves. For growth inhibition assays logarithmically growing cells were exposed to

1 The abbreviations used are: BSO, buthionine-s-1-sulfoximine; ISC, DNA-DNA interstrand cross-links; ELISA, enzyme-linked immunosorbent assay; Pt-GMP, Pt(NH3)2(dGMP); Pt-AG, cis-Pt(NH3)2(dAPG); Pt-GG, cis-Pt(NH3)2(dGpG); Pt(GMP); cisis-Pt(NH3)2(dGMP); G1ga, drug concentration resulting in 50% growth inhibition, as judged by counting tumor cell numbers; GSH, reduced glutathione; GST, glutathione S-transferase; GR, glutathione reductase; GP, glutathione peroxidase; SOD, superoxide dismutase.

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a range of drug concentrations for 1 or 24 h. After being washed with phosphate-buffered saline, cells were permitted to grow in fresh medium plus serum for a further 48 h during which the non-drug-treated cells remained in logarithmic growth. For BSO pretreatment cells were exposed to 10-20 μM BSO for 24 h prior to drug addition and during the subsequent 1-h drug treatment period. At the end of the test period monolayers were trypticized, and total cell numbers were counted using a ZBI Coulter Counter.

Uptake of [106P]Cisplatin. Radiolabeled drug uptake was measured immediately following a 1-h exposure to 2, 5, or 10 μg/ml (i.e., 6.7, 16.7, or 33.4 μM) [106P]Cisplatin (kindly supplied by Dr. H. L. Sharma, Department of Medical Biophysics, University of Manchester, England), which was prepared as previously described (19).

GSH Levels and Total GST, GR, GP, Catalase, SOD, and DT-diaphorase Activities. These measurements were made for both cell lines 3 days after initial plating while the cells were still in logarithmic growth. The assays used were as described previously (12); GSH content by the GR recycling method of Griffith (20); GST according to the procedure of Habig and Jackoby (21), using 1-chloro-2,4-dinitrobenzene (Sigma Chemicals) as the substrate; GR by the method of Horn (22); GP using Cumene hydroperoxide, according to the modified method described by Paglia and Valentine (23). For the following assays, cytosolic supernatants were prepared according to the procedure of Akman et al. (24). Catalase was then assayed by the method of Beutler (25); DT-diaphorase was assayed using the procedure of Ernster (26); and SOD was assayed using the method described by McCord and Fridovich (27). Cellular protein content was estimated using either the Lowry (28) or the Bradford (29) assay. Data were compared statistically using Student's t test.

Western Immunoblotting for GSTs. Logarithmically growing cells were harvested and lysed by sonication in 50 mM Tris-HCl, pH 7.5, and 2 mM EDTA. Cytosolic protein (150 μg/lane) was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 4.5% stacking gel and then a 14% resolving gel followed by electrophoretic transfer to nitrocellulose according to the manufacturer's directions (Bio-Rad Laboratories, Ltd., Hemel Hempstead, Hertfordshire, England). Triplicate blots were blocked in 5% nonfat milk (Marvel, Cambridge's; Premier Brands, Birmingham, England) for 30 min, as described earlier (30). These blots were then probed with rabbit antiserum directed against one of the three classes of human GST (31).

Quantitation of Platinum-DNA Adducts. For quantitation of Pt-DNA adducts logarithmically growing cells were exposed for 1 h to 10 μg/ml (33.4 μM) cisplatin dissolved in 0.9% sodium chloride solution immediately prior to use. After drug exposure the cells were washed and harvested either immediately or 18 h after incubation in drug-free medium. To provide the required number of cells for DNA isolation and to negate any possible variations in growth state, cells grown and treated on 2 or 3 separate occasions were pooled, as described earlier (13). Cell pellets were stored at -20°C. DNA was isolated from 5 x 10^7 to 10^8 cells using a combined phenol and chloroform technique. Overnight lysis of cells at 50°C with sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 4.5% stacking gel and then a 14% resolving gel followed by electrophoretic transfer to nitrocellulose according to the manufacturer's directions (Bio-Rad Laboratories, Ltd., Hemel Hempstead, Hertfordshire, England). DNA-protein cross-links and to destroy any nuclease activity. DNA was then digested to nucleotides and platinum-containing oligonucleotides as described previously (32), except that sodium azide was omitted from the incubation mixture. Purity of DNA was verified by the ratio of absorbance measurements at 260 and 280 nm. and the ratio of absorbance measurements at 260 and 280 nm. Since the occurrence of Pt-DNA adducts was quantitated per gram of DNA isolated, correction for dilution by DNA synthesis during the 18-h posttreatment incubation period was carried out using parallel cell cultures, following the precise procedure detailed earlier (13). The dilution factor was calculated as the ratio of this specific activity of DNA at 18 h compared with that value at 0 h. The apparent number of lesions at 18 h divided by the dilution factor gave a "true" number of Pt-DNA adducts.

Measurement of ISC. ISC were measured in both cell lines at 0, 14, and 24 h after a 1-h exposure to cisplatin using the alkaline elution technique of Kohn et al. (33) with minor modifications (12).

RESULTS

Cytotoxicity Assays. Dose-response curves for SK-OV-3 and TR175 cells following a 1-h exposure to cisplatin are shown in Fig. 1. Concentrations of drug reducing colony formation to 50% of control levels were interpolated to yield values of 2.60 and 0.11 μg/ml (i.e., 8.66 and 0.37 μM) for the SK-OV-3 and TR175 cell lines, respectively, indicating that the SK-OV-3 cells were approximately 24-fold more cisplatin resistant than the TR175 cells. Significant differences in cisplatin response were also noted using a growth inhibition assay by comparing G10 values (see Table 1), for either a 1-h or a 24-h drug exposure duration, although these values for the two lines differed only by a factor of approximately 7. This methodology also showed that SK-OV-3 cells were significantly more resistant than TR175 cells to carboplatin (5-fold), Adriamycin (3-fold), and etoposide (3.5-fold), while responses to vincristine and cisplatin were comparable in both lines. The media used did not appear to influence cytotoxicity since the GI0 for cisplatin (24-h exposure) for the SK-OV-3 cells was

<table>
<thead>
<tr>
<th>Compounds Tested</th>
<th>Duration of Exposure (h)</th>
<th>SK-OV-3</th>
<th>TR175</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>1</td>
<td>7.10 ± 0.55</td>
<td>1.30 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>1</td>
<td>6.50 ± 0.22</td>
<td>1.25 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cadmium chloride</td>
<td>2</td>
<td>3.20 ± 0.20</td>
<td>2.71 ± 1.21</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>2</td>
<td>32.80 ± 3.20</td>
<td>9.80 ± 2.60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vincristine</td>
<td>2</td>
<td>9.52 ± 0.45</td>
<td>7.67 ± 0.82</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2</td>
<td>667 ± 69</td>
<td>190 ± 30</td>
<td>&gt;0.05</td>
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</table>

* GI0 values were obtained from full dose-response curves evaluating four different drug concentrations in duplicate and represent the mean ± SD from experiments repeated on 3-5 occasions.
not significantly modified, being 7.83 ± 0.82 μg/ml, when these cells were grown in Ham’s F-12 as opposed to the modified Eagle’s medium.

Uptake of [195mPt]Cisplatin. Dose dependence of [195mPt]cisplatin uptake was established (data not shown), and uptake, normalized for extracellular drug concentration, proved to be significantly higher in the more resistant SK-OV-3 cells than in the TR175 cells (i.e., 14.0 ± 1.4 versus 8.9 ± 0.6 pmol/10^6 cells/μg cisplatin; P < 0.01). However, when these values were corrected for the differing cellular volumes of these cells (16, i.e., 2835 ± 26 and 2103 ± 30 μm³), uptake was only marginally higher in the more resistant cells (i.e., 5.0 ± 0.5 versus 4.2 ± 0.3 pmol/10^6 cells/μg cisplatin/unit cell volume), with the difference not reaching statistical significance (P > 0.05).

Cellular Content of GSH, GST, GR, GP, SOD Catalase, and DT-Diaphorase. Levels of total GSH and associated enzyme activities, normalized for cellular protein content, are listed in Table 2. Total GSH levels were 2.8-fold higher in the more resistant SK-OV-3 cells. However, BSO pretreatment, which resulted in depletion of GSH levels by at least 90% in both cell lines, utilizing 10 and 20 μM BSO concentrations in SK-OV-3 and TR175 cells, respectively, had only a marginal effect on the cytotoxicity of either cisplatin or carboplatin when the drugs were added to the BSO-containing cultures for the last hour of the incubation, with GSH values being reduced by only approximately 8–13% in both cell lines with either drug. The BSO concentrations used permitted 85 ± 8% cell growth in both cell lines, and attempts to use higher BSO concentrations or 48-h pretreatment durations resulted in a marked loss of proliferation in both cell lines. Consistent with their higher GSH levels, significant elevations of GR (5.2-fold) and GP (1.8-fold) activities (P < 0.05 and <0.02, respectively) were detected in the SK-OV-3 cells. Total levels of GST, however, were similar for the two cell lines, and immunoblots (data not shown) indicated that the major isozyme expressed in both of these cell lines was GST π.

Activity of SOD and DT-diaphorase were comparable in the two cell lines; however, catalase levels were significantly lower (P < 0.001) in the more resistant SK-OV-3 cells.

Induction and Repair of Platinum-DNA Adducts. The specific activity of radiolabeled DNA decreased in both cell lines during the 18-h posttreatment incubation period to approximately 90% of the values immediately following treatment. Calculated dilution factors were 0.84 ± 0.14 for SK-OV-3 cells and 0.92 ± 0.11 for TR175 cells. Total platination of DNA and distribution of adducts as a percentage of total platination in ovarian tumor cell lines measured by competitive ELISA immediately and 18 h following exposure to 10 μg/ml cisplatin for 1 h are listed in Table 3, and histograms of specific adduct levels are shown in Fig. 2. A slightly higher (1.2-fold), but not significant level of total platination was found in the more sensitive TR175 cells compared with the more resistant SK-OV-3 cells immediately after a 1-h exposure to 10 μg/ml cisplatin. Following an 18-h posttreatment incubation period, however, the level of DNA platination of the more resistant cells remained high, while that of the more sensitive TR175 cells was reduced by over 50%. This suggests that whereas the SK-OV-3 cells were apparently unable to remove most of the DNA platination under these conditions, the more sensitive TR175 cells expressed some ability to repair platinum-DNA adducts. In terms of the distribution of adducts as a percentage of the total platination (see Table 3) in both cell lines the major adduct was Pt-GG, comprising 49–55% of the total platination immediately following the drug treatment and rising marginally to 51–60% 18 h after drug removal. This suggests that additional bifunctional platination of adjacent guanines may be occurring during the period of posttreatment incubation. Indeed, the monofunctional adduct Pt-GMP comprised 13–17% of the total platination in the two lines at zero time, and by 18 h the level of this adduct had fallen in both cell lines; most of this decrease probably reflected adduct rearrangement rather than DNA repair. On a percentage basis the main difference in the distribution of adducts in these two cell lines was the considerably lower initial proportion (12% versus 26%) of the adduct Pt-AG, although similar proportions remained in both cell lines after the 18-h posttreatment incubation period (16–17%). Both cell lines appeared to be proficient in the repair of this Pt-AG adduct, with approximately 30% being removed during the 18-h posttreatment incubation period. In the more sensitive TR175 cells there was also a higher percentage of Pt(GMP), both initially and after the 18-h posttreatment incubation. In addition, there were differences in the removal of this adduct between the two lines; TR175 cells appeared to be proficient in this respect, while none of this adduct was removed by the SK-OV-3. This deficiency in removal of the platinated DNA was also particularly marked in these SK-OV-3 cells with respect to the major Pt-GG adduct, although more than 50% of this lesion was removed by the more sensitive TR175 cells.

Table 2 Glutathione levels, related enzyme activities, and certain antioxidant and xenobiotic-metabolizing enzyme activities

<table>
<thead>
<tr>
<th>Parameters measured</th>
<th>SK-OV-3 cells</th>
<th>TR175 cells</th>
</tr>
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<tbody>
<tr>
<td>GSH (nmol)</td>
<td>91.1 ± 14.0</td>
<td>33.1 ± 7.3</td>
</tr>
<tr>
<td>GR (nmol NADPH/min)</td>
<td>104.5 ± 17.5</td>
<td>20.2 ± 3.2</td>
</tr>
<tr>
<td>GST (nmol CDNB/min)</td>
<td>117.0 ± 19.1</td>
<td>86.3 ± 25.7</td>
</tr>
<tr>
<td>GP (nmol NADPH/min)</td>
<td>15.7 ± 2.2</td>
<td>8.7 ± 1.6</td>
</tr>
<tr>
<td>SOD (μg)</td>
<td>1.7 ± 0.47</td>
<td>1.36 ± 0.01</td>
</tr>
<tr>
<td>Catalase (μmol H₂O₂/min)</td>
<td>3.46 ± 0.11</td>
<td>7.99 ± 0.32</td>
</tr>
<tr>
<td>DT-diaphorase (nmol NADPH/min)</td>
<td>508 ± 31</td>
<td>504 ± 11</td>
</tr>
</tbody>
</table>

* All of the values were normalized for cellular protein content and expressed per mg. The values quoted are the mean of at least 3 experiments in which quadruplicate estimations were carried out ± SD.

* P < 0.05.
* P < 0.02.
* P < 0.001.

4559
Since the antiserum W101 is unable to distinguish between Pt(GMP), derived from ISC or long-distance intrastrand cross-links formed by binding of cisplatin to nonadjacent guanines in the sequence GpG(pX)pG, alkaline elution was used to quantitate ISC. Table 4 shows the frequency of ISC in the two cell lines at 14 and 24 h after a 1-h exposure to a range of cisplatin concentrations. Values obtained at zero time points were not included since these were generally less than 1.0 rad-equivalents and so were difficult to quantitate accurately. ISC induction was dose dependent in both lines, but when the levels at 14 h were compared on an equimolar basis, a higher frequency (i.e., 1.6–2.8-fold) was observed in the more resistant SK-OV-3 cells. In both cell lines there was a significant loss of cross-links by 24 h, indicative of substantial repair of ISC by both of these ovarian tumor cell lines.

**DISCUSSION**

In these studies we have attempted to establish whether the marked differences in *in vitro* sensitivities to cisplatin exhibited by two human ovarian carcinoma cell lines, established from tumors from patients who had both received non-cisplatin-based chemotherapy, could be explained in terms of differential modulation of cisplatin cytotoxicity. Mechanisms generally associated with resistance to cisplatin have been investigated and the following parameters have been quantitated: drug uptake; glutathione levels and associated enzyme activities; and extent of platinum-induced DNA damage and repair. These studies differ from many previously described in the literature involving the comparison of these various parameters expressed by parental (sensitive) cell lines and various drug-selected cisplatin-resistant sublines. This work may provide information as to whether comparable mechanisms are operating in cells that were not previously exposed to cisplatin and yet appear to be inherently cisplatin resistant. These studies serve to complement and extend our earlier investigations examining the differential cisplatin sensitivities of two human prostate cell lines (34) and those expressed by human bladder carcinoma and teratoma cell lines (13, 19).

We reported previously that there were no major differences in the fundamental growth characteristics of these two ovarian tumor cell lines, apart from SK-OV-3 cells having a 30% larger overall cellular volume and an increased colony-forming efficiency in semisolid agar and readily forming xenografts in nude mice, unlike the TR175 cells (16). In terms of drug-selected cisplatin-resistant sublines, there are reports of slower growth rates and/or population doubling times (35–42), although others have not identified any significant differences in these properties (12, 15, 43–46). The difference in sensitivity to the cytotoxic action of cisplatin expressed by these two ovarian tumor cell lines has been identified using both clonogenic and growth inhibition assays, although a much larger difference was detected using the cell survival assay as opposed to the short-term cell counting procedure. This may purely reflect the greater sensitivity and precision of the cloning method. SK-OV-3 cells generally proved more resistant to all the antitumor drugs tested than the TR175 cells, although the differences were not significant for vincristine. Absence of cross-resistance to vincristine has been generally reported in cisplatin-resistant cells (37, 47, 48). Similarly, full or partial cross-resistance to carboplatin has been identified (12, 15, 37, 38, 41–44, 46, 48) as well as some partial cross-resistance, where tested, to melphalan and mitomycin C (35, 37, 43, 48, 49). In terms of cross-resistance to Adriamycin, cisplatin-resistant sublines generally either retain full sensitivity to the drug (15, 16, 43) or, as with the SK-OV-3 cells, show limited cross-resistance (37, 38, 42, 46, 48). The resistance to etoposide in SK-OV-3 cells, however, appeared unusual since cisplatin-resistant L1210 cells proved more sensitive (50), while a resistant immunocytoxin line retained its parental cell sensitivity to this drug (48).

Resistance to cisplatin and drug-selected sublines of both murine and human origin has been associated with significantly decreased cisplatin accumulation (8, 15, 46, 47, 51–58). This is by no means a universal finding (37, 41, 46, 57–60), however, and significant differences were not apparent in terms of uptake between the SK-OV-3 and TR175 cell lines, once corrections were made for their differing cellular volumes. Indeed, the frequent lack of correlation shown between the extent of uptake and degree of cisplatin sensitivity (13, 44, 46, 47, 53, 55, 61, 62) and the observation that reduced accumulation in somatic cell hybrids between sensitive and cisplatin-resistant L1210 cells was a recessive trait (55) serve to illustrate that any role for differential drug uptake in the expression of cisplatin resistance remains questionable. Renewed interest in this topic is likely, however, with the recent identification of a membrane glycoprotein, distinct from the multidrug resistance-associated
cisplatin and platinum-DNA adducts in ovarian carcinoma cells

P-glycoprotein, being overexpressed in cisplatin-resistant murine lymphoma sublines (63). Published reports also indicate that reduced drug uptake may (56) or may not (64–66) be reflected in a reduced level of drug bound to DNA. However, some more recent detailed studies of cisplatin accumulation (67) have concluded that diminished retention, as opposed to uptake, of platinum in the rapidly effluxing pool of resistant ovarian tumor cells is a major determinant of decreased cisplatin accumulation in these cells.

A reduction in the interaction between cisplatin and DNA might also be caused by an elevation in GSH and the activity of related enzymes, as well as increased levels of the cytosolic protein metallothionein (9, 10). GSH is an intracellular non-protein compound considered to play a key role in drug detoxification. BSO depletes GSH levels normally maintained by the enzyme GR (cf. Ref 9). Elevated GSH levels have been shown in certain cisplatin-resistant human ovarian carcinoma lines (12, 19, 37, 43, 45, 57, 61, 68) as well as those derived from other tumor types (37, 42, 44, 51, 57, 65, 69). Again, however, this is not a universal finding (15, 46, 51, 56), and Richon et al. (55) showed that increased levels of GSH were a recessive trait in cisplatin-resistant L1210 cells. Similarly, BSO-mediated GSH depletion has been shown to restore cisplatin sensitivity in certain resistant sublines, at least in part (9, 54, 69), but not in others (44, 55, 61). More recent evidence from Andrews et al. (46, 70), however, emphasizes the need for prolonged BSO treatment prior to and during cisplatin exposure for effective resistance reversal. Furthermore, these authors caution that the role of GSH in intracellular cisplatin metabolism may vary between cell lines (70). Cisplatin resistance in SK-OV-3 cells certainly appears to be associated with increased GSH levels, but our experiments did not reveal any marked increase in cisplatin sensitivity in SK-OV-3 cells following depletion of GSH by BSO. Increased activities of GR and GP, but not GST, also appear to be characteristic of these SK-OV-3 cells. We have recently reported that in a wide range of human tumor cell lines there was a significant correlation between cisplatin sensitivities, as judged by clonogenic assay, and levels of total GSH and activities of GP but not levels of either GR or GST activities (71). Increased GR and GP but not GST activities have also been described in a cisplatin-resistant human bladder carcinoma subline (12). However, others have reported no changes in these enzyme activities (9, 37, 41, 43, 44, 57, 72), although both increased (41, 44, 45) and decreased (57) GST levels have been recorded. Reduced catalase activity in the SK-OV-3 cells may be associated with their relative resistance to Adriamycin and etoposide, consistent with the reported decrease in activity in Adriamycin-resistant MCF-7 cells (24).

Metallothioneins are intracellular proteins involved in metabolizing heavy metals such as zinc, cadmium, and copper (73) and, owing to their high content of reactive thiol groups, are prime potential targets for agents such as cisplatin (53). Increased metallothionein levels have been reported in cisplatin-resistant human head and neck squamous carcinoma, melanoma, small cell carcinoma, large cell carcinoma, ovarian carcinoma, and prostate tumor cell lines (34, 44, 74, 75). In apparent contrast to this, Fujiwara et al. (72) identified reduced levels of this protein in a cisplatin-resistant lung cancer cell line. However, a recent report (76) that cisplatin increased metallothionein levels in drug-sensitive L1210 cells but not in drug-resistant cells led to the conclusion that metallothioneins are not responsible for their resistance to cisplatin. Indeed, this was the conclusion reached earlier by Eastman et al. (50), when reporting increased sensitivity to cadmium chloride in L1210 cisplatin-resistant cells, and reiterated more recently by Schilder et al. (10) with respect to human ovarian cancer cell lines. Our results also do not implicate a role for these proteins in the more resistant SK-OV-3 cells since significant differences in cadmium chloride sensitivities between these two cell lines were not identified.

Although it is generally accepted that DNA is the major target for the cytotoxic action of cisplatin (for reviews, see Refs. 11, 50, 77), the platinum-DNA adduct(s) responsible for the antitumor activity of cisplatin remains to be identified. Data from the majority of published studies have indicated a positive correlation between the extent of ISC formation and cisplatin cytotoxicity (for a review, see Ref. 78). A decreased formation of ISC has been described in cisplatin-resistant fibroblasts (79), murine L1210 cells (80), and a range of human tumor sublines established from small cell (37) and non-small cell lung cancer (58), head and neck squamous cell carcinoma (44), bladder carcinoma (12, 19), and colon carcinoma (42). These correlations of course do not imply a causal relationship. Indeed, in apparent contrast to this general trend in the present study more ISC were formed in the more resistant SK-OV-3 cells, a result similar to that reported earlier when two testicular teratoma lines with differing cisplatin sensitivities were compared (13). These lesions appeared to be readily repaired by both ovarian lines, however, in contrast to the lack of repair ability noted in the cisplatin-hypersensitive teratoma lines (13). Differential repair of these ISC lesions has also been reported in various other model systems, but while this has been shown to be enhanced in certain more resistant sublines (15, 81), there have also been reports of reduced rates of removal in resistant cells (12), implying increased tolerance of DNA damage or even similar rates of repair in both sensitive and resistant cell lines (58). However, since ISC comprise less than 2% of the total amount of DNA platination, more recently a number of groups have directed their efforts toward quantitating intrastrand adduct formation using a range of newly developed antisera (32, 82, 83).

There is indirect evidence suggesting a correlation between the formation of intrastrand diammineplatinum adducts in leukocyte DNA with a favorable disease response to cisplatin-containing chemotherapy in ovarian cancer patients (84). However, these results need to be tempered by other reports of large interindividual variations in the formation of intrastrand cross-links in the leukocytes of patients treated with cisplatin (85, 86). Terheggen et al. (87), using quantitative immunocytochemistry of platinum-DNA binding, also recently reported a close correlation between initial levels of cisplatin-DNA binding and sensitivity to cisplatin. However, the majority of published data tend to argue against this with similar adduct levels being reported, both in the present study and in studies of certain sensitive and resistant tumor cell lines including those derived from human colon carcinoma cells (42), a rat IgM immunocytoma (59), and an ovarian carcinoma. Furthermore, evidence of increased adduct levels in the more resistant cell lines immediately after cisplatin exposure have also been published (13, 15).

Enhanced repair of cisplatin-induced damage is frequently evoked as a resistance mechanism. We suggested earlier (13) that the inability of a human testicular teratoma cell line (SuSa) to repair platinated DNA may account for its hypersensitivity.
to cisplatin and more recently showed increased repair capacity in a cisplatin-resistant SuSa subline (15). However, in the present study it is the more resistant SK-OV-3 cell line which appears to be repair deficient, especially in terms of the major Pt-GG adduct. This clearly contrasts with other reports of increased repair, as judged by removal of adducts by resistant cells (11, 14, 65, 75), monitoring of unscheduled DNA synthesis (60), and increased activities of DNA polymerase β (88). An enhanced DNA repair capacity has also been suggested by transfection studies using cisplatin-damaged plasmids with L1210 cells (89). However, we also reported earlier (90) that the more sensitive TR175 cells replicated cisplatin-treated adenovirus to a lower extent than the SK-OV-3 cells, implying that the TR175 cells may be repair deficient. However, the present study contradicts this proposal, indicating, as others have noted (cf. Ref. 90), that the inability of a cell to reactivate a damaged virus is not always associated with a measurable repair deficiency of total genomic DNA. In contrast to the above reports, no differences in the kinetics of excision of adducts were recently reported in sensitive and resistant colon carcinoma cell lines (42), and Hospers et al. (91) have concluded that there was no evidence in their small cell lung carcinoma line that cisplatin resistance was based on differential repair of the Pt-GG, Pt-AG, Pt(GMP), or Pt-GMP adducts. They therefore speculated that another adduct, not detected by their immunological method, was involved, and this could possibly be GSH-Pt-DNA, as suggested by Eastman (92). However, before altered repair of drug-induced damage can be ruled out as an important mechanism in human cancers, new more direct approaches to studying this phenomenon need to be developed. In this respect the cell free repair system developed by Wood et al. (93) may prove valuable in this respect.

In the present study, therefore, resistance to cisplatin in SK-OV-3 cells appears to be associated with increased levels of GSH and certain associated enzyme activities, which appear, however, to permit total platination levels of DNA comparable with those achieved initially in the more sensitive TR175 cell line. However, since these SK-OV-3 cells proved deficient in the removal of these adducts with time, they appear to be able to tolerate this residual damage and may be able to replicate on a damaged template. This increased tolerance to unrepaired lesions in the DNA has been reported in cisplatin-resistant L1210 cells (94) and in cisplatin-resistant sublines derived from a human bladder carcinoma and a human testicular teratoma (12, 15). Mechanisms by which cells could become tolerant include alterations in DNA replication and postreplication repair (11). In addition, since the techniques used in this study only measure DNA damage and its repair in the overall genome, heterogeneity of drug-induced DNA damage and repair in specific genomic regions, as recently reported in relation to cisplatin (95), cannot be ruled out.

The relative sensitivity of line TR175 to cisplatin, however, remains to be explained. These TR175 cells accumulate cisplatin at a rate similar to that of the more resistant SK-OV-3 cells, and total levels of platination were also comparable. Their GSH levels, however, lower than those of the SK-OV-3 cells, are still considerably higher than those reported in other human tumor cell types not proving particularly sensitive to the cytotoxic effects of cisplatin (71). Furthermore, this marked sensitivity to cisplatin cannot be explained in terms of a defective ability to remove platinum-induced ISC or intrastrand cross-links. These TR175 human ovarian tumor cells therefore appear to show properties similar to those previously described by Rawlings and Roberts (64) in a wild-type Walker rat carcinoma cell line and so provide another example indicating that sensitivity to DNA damage is not automatically associated with deficient DNA repair.

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Anomalous Relationship between Cisplatin Sensitivity and the Formation and Removal of Platinum-DNA Adducts in Two Human Ovarian Carcinoma Cell Lines in Vitro

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