Antitumor Activity and Biochemical Effects of Aphidicolin Glycinate (NSC 303812) Alone and in Combination with Cisplatin in Vivo

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ABSTRACT

Aphidicolin, an inhibitor of DNA polymerases α and β, is cytotoxic in vitro against tumor cells. The poor solubility of aphidicolin has led to the development of aphidicolin glycinate (AG; NSC 303812), a water soluble ester currently in early clinical trials. The antitumor activity of AG was investigated in a series of transplantable murine tumors in vivo. The drug demonstrated activity against the i.p. implanted B16 melanoma, producing maximum increased life spans of 75% following i.p. administration every 3 h for three doses on days 1–9. Treatment schedules involving both single injections per day on days 1–9 and multiple injections per day on days 1, 5, 9, and 13 were less effective, indicating that this antitumor activity is schedule dependent. Similarly, greater activity was observed against the i.p. M5076 sarcoma when three daily injections were given on days 1–9 (57% increased life span) than with a single injection either on days 1–9 (36% increased life span) or on days 1, 5, 9, and 13 (inactive). Further scheduling studies in the s.c. M5076 sarcoma model showed that a 7-day infusion was superior to both a 24-h infusion and a 7-day course of three bolus treatments per day. On the assumption that DNA polymerase inhibition is the basis for this antitumor activity, inhibition of DNA synthesis in BALB/c x DBA/2 F1 mice was investigated by measuring incorporation of [3H]thymidine (20 μCi, i.v.) into DNA of spleen and jejunum. At 2 h after administration of AG, inhibition of DNA synthesis was dose dependent (median inhibitory dose, 60 mg/kg in both tissues) and was >99% at 300 mg/kg. The inhibition was rapid in onset; AG (100 mg/kg i.p.) produced maximal (>98%) inhibition in both tissues at 30 min. Recovery occurred in the intestine within 16 h; in spleen recovery was delayed to 24 h, and was followed by a rebound incorporation at 48 h (203%). A comparison of the inhibition of thymidine incorporation in tumor cells (B16 melanoma and P388 leukemia) and normal jejunum revealed no significant differences in the extent of inhibition or the rapidity of recovery in these tissues. The rapid recovery of DNA synthesis inhibition supports the use of prolonged infusion schedules in clinical trials, but the lack of evidence of selectivity for tumor cells suggests that AG may be of limited therapeutic value as a single agent. Thus, we evaluated AG in combination with cisplatin in an in vivo model of cisplatin refractory human ovarian cancer. In this model system, AG as a single agent had minimal activity whereas its use in bolus form at 30 mg/kg/dose every 3 h for 4 doses with cisplatin (5 or 8.3 mg/kg) at 90 min after the second AG injection was markedly superior to cisplatin alone. Furthermore, a continuous 7-day infusion of 150 mg/kg/day with cisplatin (8.3 mg/kg) on the second day of the AG infusion was equivalent in efficacy to the maximum tolerated dose of cisplatin (14 mg/kg). These data, in combination with information on the sustained levels of AG which may be achieved in patients support the clinical development of combination therapy with AG and cisplatin/carboplatin in platinum refractory patients.

INTRODUCTION

Many of the most active anticancer drugs are thought to have DNA as their ultimate target. The interaction of electrophilic drugs with DNA may result in various forms of DNA damage, the repair of which often involves an excision step in which the monooxaduct, or damaged base or nucleotide is removed, along with undamaged nucleotides on either side of the lesion, followed by filling and reannealing of the gap thereby produced (1). The latter steps require the joint action of DNA ligase and DNA polymerases (2). Aphidicolin, a fungal derivative that inhibits DNA polymerases α and β (3–7), has recently been introduced for clinical studies as the glycinate derivative (8).

Aphidicolin is a diterpene antibiotic derived from cultures of Cephalosporium aphidicolum, and is a specific reversible inhibitor of DNA polymerase α and β. A series of studies demonstrated that aphidicolin inhibits DNA polymerase α, but has no effect on DNA polymerases β or γ, reverse transcriptase, or prokaryotic DNA polymerase II (3, 9, 10). It appears that aphidicolin binds reversibly to the enzyme, not to DNA, and that the drug is selectively toxic in S phase. Deoxynucleotides do not abrogate the growth inhibition produced by aphidicolin, indicating that the drug does not act via inhibition of nucleotide synthesis (11). The drug was a potent selective inhibitor of thymidine incorporation with complete and immediate inhibition of the incorporation of labeled thymidine into DNA at 1 μM, but no inhibition of uridine incorporation into RNA, or of leucine incorporation into protein, even at 1 mM (11).

Kinetic analysis of the interaction of aphidicolin and DNA polymerase suggests that inhibition is competitive with respect to dCTP, noncompetitive with respect to dATP, and dGTP, and dTTP (12, 13). It is suggested that the binding of aphidicolin to the DNA polymerase occurs at a site overlapping that for dCTP, and that conformational changes induced by its binding may inactivate the enzyme (13).

AG5 inhibits the growth of P388 leukemic cells grown in vitro, with a concentration that inhibits growth by 50% for a 48-h exposure of 0.38 μM. The concentrations required for growth inhibition are markedly higher with shorter drug exposure times, indicating that the cytotoxic action of aphidicolin is highly schedule dependent (11).

The role of the DNA polymerases in mammalian nucleotide excision repair has suggested the value of aphidicolin to study and influence this process for clinical benefit. Of direct relevance to cancer chemotherapy, it has been shown that aphidicolin inhibits repair of cisplatin induced DNA damage as measured by unscheduled DNA synthesis (14). Furthermore, multiple groups have reported aphidicolin augmentation of cisplatin cytotoxicity in vitro (16). It has been presumed that this effect is the result of blockade of repair of DNA lesions critical to cell survival.

In this paper we present evidence for the in vivo cytotoxicity of AG, and describe its schedule dependency in vivo. We describe the biochemical effects of aphidicolin administration on DNA synthesis in normal tissues and in murine tumors in BALB/c x DBA/2 F1 (here-
after called CD2F1) mice. This information, combined with data supportive of a role for DNA repair in resistance to cisplatin mediated in part by DNA polymerase, was used to design subsequent experiments using AG and cisplatin combinations in an in vivo model of cisplatin refractory human ovarian cancer.

MATERIALS AND METHODS

Mice, Murine Tumors, and Chemicals. Mice and tumors were obtained through the Biological Testing Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. AG was obtained through the Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. Alzet pump Models 2024S and 2001, purchased from Alza Corporation (Palo Alto, CA), were used to administer 1-day and 7-day s.c. infusions of AG, respectively. 6-[3H]Thymidine (100 μCi/ml) was obtained from New England Nuclear, Amersham, MA. All other reagents were purchased from Sigma.

Experimental Chemotherapy of Murine Tumors. Antitumor evaluations were conducted through DTP's in vivo screening program according to published NCI protocols (17-19). The mice were housed in holding rooms until their weights were appropriate (minimum 17 and 18 g for males and females, respectively) for tumor transplantation and experimentation. They were provided with food and water ad libitum and maintained on a 12-h light, 12-h dark cycle. For evaluation of the sensitivity of transplantable murine tumors to AG, CD2F1 mice were inoculated with either 10^6 L1210 leukemia ascites cells i.p. or with 10^6 P388 leukemia cells i.p. or s.c., and C57BL/6 × C3H F1 (hereafter called B6C3F1) mice were inoculated with either 0.5 ml of 1:10 B16 melanoma brei i.p., 10^6 M5076 sarcoma ascites cells i.p. or s.c., and 5 × 10^5 M5076 sarcoma cells s.c. Tumor implantation day was designated day 0. Treatment with AG dissolved in saline, or saline containing 2% alcohol, was initiated 24 h later. In each experiment, AG was tested at several dosage levels, and each dose was administered to 6 or 10 mice. In each experiment, the number of tumor- control mice varied between 16 and 50 in accordance with NCI protocol 9 (17).

In situ measurements of tumor length and width were taken twice a week for evaluation of the response of the s.c. implanted M5076 sarcoma tumors to AG treatment. Tumor size was converted to weight by using the formula

\[ \text{Tumor wt (mg)} = \frac{\text{length} \times \text{width}^2}{2} \]

where length and width were expressed in mm. Antitumor activity was assessed on the basis of percentage of T/C and tumor growth delay. Percentage of treated/control was calculated by dividing the median treated tumor weight on day 12 by the median control tumor weight on the same day and multiplying by 100. Growth delay was expressed as a percentage by which the treated tumor weight was delayed in attaining 1000 mg compared to the controls by using the formula

\[ \frac{T - C}{C} \times 100 \]

where T and C are the median times (days) to attain 1000 mg for the treated and control groups, respectively.

For all other antitumor studies, animals were retained for life span determinations. Median life spans were calculated from grouped median survival times and the percentage of ILS was calculated as

\[ \left( \frac{100 \times \text{median survival time of treated mice}}{\text{median survival time of control mice}} \right) - 100 \]

Comparisons of survival curves for particular treatments were performed by using the log-rank test.

Inhibition of DNA Synthesis. To study the effects on normal tissues, male CD2F1 mice (about 25 g) were given injections i.p. of 10% AG (w/v). Controls received an equivalent volume of normal saline i.p. At a defined interval following drug injection, 6-[3H]Thymidine at 0.8 μCi/g was administered i.v. After 20 min, mice were killed by cervical dislocation. The spleen was placed immediately in liquid nitrogen; the jejunum was removed, washed in cold saline, divided, washed again, and frozen in liquid nitrogen. Frozen tissues were homogenized and extracted in 0.3 M PCA. The acid-precipitable material was washed twice in 0.3 M PCA, then resuspended in 2 M PCA, and incubated for 1 h at 95°C to hydrolyze DNA. The sample was then chilled, and protein was removed by centrifugation. Radioactivity in 50 μl of the supernatant was determined by liquid scintillation counting.

To investigate the effects on tumor tissues, CD2F1 mice were inoculated i.p. with P388 leukemia, C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) mice were inoculated i.p. with B16 melanoma. After 6 days (P388) or 13 days (B16), mice were treated with AG, followed by i.v. injection of labeled thymidine as described above. After the animals were killed, the jejunum was removed and processed as described previously. Mice bearing P388 leukemia were given injections i.p. of 5 ml ice-cold saline initially to harvest the cells, which were centrifuged, weighed, and extracted immediately with PCA as described. In mice bearing B16 melanoma, the tumor was removed, frozen in liquid nitrogen, and stored at −70°C for later processing. The procedure for extractions were as described above.

In Vivo Model of Cisplatin-resistant Human Ovarian Cancer. The OVCAR-3 cell line, which was derived from the tumor of a patient refractory to combination chemotherapy with cisplatin, cyclophosphamide, and Adriamycin, when injected i.p. into female athymic nude mice, produces i.p. carcinomatosis and ascites characteristic of clinical ovarian cancer (20). Animals reproducibly die from complications of this i.p. disease and the malignant ascites may be harvested and used to transplant the tumor to subsequent hosts. This in vivo model system has been used for evaluation of new drugs (21), drug analogues (22), and modulators of alkylating agent resistance (23).

RESULTS

AG Antitumor Activity in Murine Tumor Models. AG demonstrated marginal to moderate activity in four i.p. implanted murine tumor models following administration on an i.p. daily for 9-day treatment regimen involving single bolus injections per day. Maximum ILS values ranged from 33 to 38% in four B16 melanoma experiments, 27 to 40% in three P388 leukemia studies, and 27 to 53% in three L1210 leukemia experiments. A maximal ILS of 36% was attained in a single M5076 sarcoma study using the same treatment regimen, and no activity (ILS < 25%) was observed in a separate experiment involving single treatments of AG on days 1, 5, 9, and 13 (data not shown). AG was more effective in two of the same four tumor models when multiple doses per treatment day were evaluated (Table 1). In the i.p. B16 melanoma model, maximum ILS values of 76 and 75% were attained in two experiments when a 40-mg/kg dose was administered every 3 h 3 times a day on days 1–9 (total dose/day, 120 mg/kg). The maximum ILS values attained in the same two studies following single injections of 75 and 50 mg/kg on the same treatment days were, respectively, 33 and 36%. Similarly, the more intense treatment regimen was more effective in the i.p. M5076 sarcoma model producing maximum ILS values of 57 and 56% compared to a 36% ILS with the single injection per day schedule. However, no difference in activity was noted when the single and 3 times daily treatment regimens were compared in the i.p. P388 and L1210 leukemia models, and neither regimen was effective in the s.c. P388 leukemia model.

Schedule Dependency of AG Antitumor Activity. The effects of schedule on the antitumor efficacy of AG were explored further in the i.p. B16 melanoma model (Table 2). AG administered i.p. was not highly effective on any of the bolus treatment schedules. However, when the effects of three paired treatments involving single and multiple injections per day were compared (single and every 3 h for 8 doses on day 1 only, single and every 3 h for 3 doses on days 1–9, and single and every 3 h for 8 doses on days, 1, 5, and 9), the regimens involving multiple treatments per day were more active than those involving single treatments per day and more drug was tolerated. These studies were extended to evaluate infusion schedules in the s.c. M5076 sarcoma model (Table 3). The most effective schedule was the 7-day infusion. With the 300-mg/kg/day infusion rate, no tumors had...
The time course of DNA polymerase inhibition was investigated by using a 10% lethal dose of AG, 100 mg/kg. The onset of inhibition was rapid, with maximal inhibition by 30 min, and recovery at 16 (jejunum) to 24 (spleen) h (Fig. 2). In spleen a rebound increase in activity (to 203%) was observed at 48 h. These data confirm in vivo inhibition of DNA polymerase by aphidicolin is rapidly reversible, presumably with elimination of the drug from the circulation.

Evidence for selective inhibition of DNA synthesis in tumor versus normal tissue was sought with the use of two models: P388 leukemia implanted i.p. in CD2F1 mice, and B16 melanoma implanted i.p. in B6D2F1 mice. The normal tissue sampled in each case was jejunum. As is evident from the dose-response curves (Figs. 3 and 4), no significant differences between the characteristics of inhibition of thymidine incorporation could be discerned between tumor cells and normal jejunum. Recovery of DNA polymerase activity followed a similar time course in normal jejunum and in B16 melanoma, while activity was restored more rapidly in P388 leukemia cells (Fig. 5 and 6).

In Vivo Model of Cisplatin-resistant Human Ovarian Cancer. Based on the role of DNA repair in cisplatin resistance, the combination of AG and cisplatin was next evaluated in an in vivo model of human ovarian cancer (Fig. 7). This model is based on the OVCAR-3 ovarian cancer cell line which was derived from the ovarian tumor of an ovarian cancer patient who had failed treatment with cisplatin, Adriamycin, and cyclophosphamide. This cell line is 3- to 5-fold less sensitive to cisplatin than a “benchmark” ovarian cancer cell line, A2780, derived from an untreated patient (24). Based on the data presented above indicating the importance of schedule dependence on efficacy, AG was initially evaluated by using multiple i.p. doses (every 3 h for 4 doses on day 4 posttumor implantation and every 3 h for 2 doses on day 5, 30 mg/kg/dose). When used in combination with

<table>
<thead>
<tr>
<th>Tumor system</th>
<th>Control median survival time (days)</th>
<th>Treatment i.p. days 1–9</th>
<th>Treatment i.p. every 3 h for 3 doses days 1–9</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 melanoma i.p.</td>
<td>20.2</td>
<td>50, 27.0</td>
<td>33, 40</td>
</tr>
<tr>
<td>M5076 sarcoma i.p.</td>
<td>16.9</td>
<td>75, 23.0</td>
<td>36, 40</td>
</tr>
<tr>
<td>P388 leukemia i.p.</td>
<td>23.5</td>
<td>60, 32.0</td>
<td>36, 20</td>
</tr>
<tr>
<td>P388 leukemia s.c.</td>
<td>11.3</td>
<td>60, 14.4</td>
<td>27, 20</td>
</tr>
<tr>
<td>L1210 leukaemia i.p.</td>
<td>15.6</td>
<td>120, 17.7</td>
<td>4 Neg, 40</td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>100, 12.4</td>
<td>27, 40</td>
</tr>
</tbody>
</table>

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Table 3: Effects of schedule on activity of AG against s.c. M5076 sarcoma in mice

Groups of 10 B6C3F1 mice (19 controls) were implanted with 5 × 10^6 M5076 sarcoma cells s.c. on day 0. Treatment s.c. with a solution of AG in saline was initiated on day 1 according to the schedules outlined below. Infusions were administered by means of Alzet minipump Models 2011 and 2024S for the 7-day and 24-h infusions, respectively.

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Optimal dose (mg/kg/day)*</th>
<th>Total dose (mg/kg)</th>
<th>Net body wt change (%)</th>
<th>Day 12 T/C†</th>
<th>Time to reach 1000 mg (days)</th>
<th>Growth delay: C T – C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (untreated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every 3 hr for 3 doses, days 1–7</td>
<td>100</td>
<td>2100</td>
<td>+1.8</td>
<td>16</td>
<td>22.6</td>
<td>39</td>
</tr>
<tr>
<td>7-day infusion, days 1–7</td>
<td>300</td>
<td>2100</td>
<td>–2.8</td>
<td>16</td>
<td>22.6</td>
<td>39</td>
</tr>
<tr>
<td>24-h infusion, days 1–7</td>
<td>533</td>
<td>533</td>
<td>+0.1†</td>
<td>19</td>
<td>20.0</td>
<td>23</td>
</tr>
</tbody>
</table>
between the group treated with cisplatin alone at 5.0 mg/kg and cisplatin at 5.0 mg/kg with AG was made. Again, survival was superior for the AG-treated group ($P < 0.005$).

Evidence that a 7-day infusion of AG as a single agent was superior to repeated bolus injections (Table 3) supported the development of this approach in the OVCAR-3 model. Therefore, a second experiment was conducted in which AG was evaluated by a s.c. 7-day infusion at its maximally tolerated dose of 300 mg/kg/day as determined in the M5076 study (Table 3). This treatment, initiated on day 4, had no benefit as monitored by increase in life span in this model (data not shown). Single doses of 14, 8.3, and 5 mg/kg cisplatin were administered i.p. on day 5. Optimal activity was observed with the 8.3-mg/kg dose; median life span of dying mice was increased from 33 to 95 days, and 4 of 10 mice survived until the experiment was terminated on day 147. The use of these agents in combination at the doses and schedule described above was toxic, resulting in a decrease in median survival from that of the untreated controls. Thus, a modified combination study was conducted in which the infusion rate of AG was decreased to 150 mg/kg/day. In this confirmatory experiment, the combination of AG and cisplatin (8.3 mg/kg) was as efficacious as cisplatin alone at its maximum tolerated dose (14 mg/kg), based on the

cisplatin (8.3 and 5.0 mg/kg), the cisplatin dose was injected 90 min after the second AG dose. In this study AG had a minimal effect on life span, i.e., 26%. Cisplatin alone showed benefit (5 mg/kg, 83% increase in life span and 8.3 mg/kg, 95% increase in life span). In the case of AG plus cisplatin, 5 mg/kg, 8 of 10 animals were alive at the termination of the experiment on day 129 (median survival untreated controls, 48 days) and in the case of AG plus cisplatin (8.3 mg/kg), 6 of 10 animals were alive at the termination of the experiment (Fig. 7).

We used the log-rank test to test for significance between the survival rates for the group treated with cisplatin (8.3 mg/kg) and that treated with cisplatin (5.0 mg/kg) with AG. The latter group had strikingly superior survival ($P < 0.004$). A similar comparison be-

![Fig. 1. Effects of administration AG i.p. at various doses upon the incorporation of $[^3H]$thymidine into DNA of small intestine and spleen of CD2F1 mice (6–10/group) 2 h after drug administration.](image1)

![Fig. 2. Effects of AG 100 mg/kg i.p. upon DNA incorporation of $[^3H]$thymidine at various times after drug administration in CD2F1 mice (6–10/group).](image2)

![Fig. 3. Effects of AG at various doses i.p. upon DNA incorporation of $[^3H]$thymidine in normal jejunum and i.p. B16 melanoma in B6D2F1 mice. Tissues were sampled 2 h after drug administration.](image3)

![Fig. 4. Incorporation of $[^3H]$thymidine into DNA of normal jejunum and i.p. P388 leukemia in CD2F1 mice treated with AG at various doses. Tissues were sampled 2 h after drug administration.](image4)
their major mechanism of cytotoxicity (e.g., methotrexate, hydroxyurea) are similarly schedule dependent, consistent with cell cycle arrest as a requirement for cytotoxicity. Why this mechanism should confer a selective action against tumors has never been clear: the results of this study imply that the selectivity of a direct inhibition of DNA polymerase is rather marginal. Such a conclusion is supported by the thymidine incorporation studies. Inhibition of thymidine incorporation into DNA was observed in both splenic and jejunal tissue. Inhibition was dose dependent and was >98% at 300 mg/kg of AG. The median inhibitory dose for inhibition of DNA synthesis in these tissues was 60 mg/kg. An examination of the patterns of recovery suggested that recovery was delayed in spleen over jejunal tissue. This observation might be of some importance in that the duration of recovery could have a significant impact upon selectivity, especially for combination studies of AG with other agents.

These data on AG single agent efficacy, coupled with the potential to increase the activity of cisplatin, were used to design combination experiments with cisplatin and AG in our in vivo model of cisplatin-resistant ovarian cancer. It was noteworthy that efforts to optimize dose and schedule of the combination (AG 7-day infusion at 150 mg/kg/day and cisplatin at 8.3 mg/kg) resulted in a survival benefit equivalent to cisplatin alone administered at its maximum tolerated dose of 14 mg/kg, and it is apparent that with less optimal AG administration the combination of AG with cisplatin (5.0 or 8.3 mg/kg) was markedly superior to cisplatin alone at equivalent doses. The Phase I clinical trials of AG clearly show that prolonged steady-state levels of AG of at least 3 μg/ml can be achieved (8), and this concentration has been shown to maximally inhibit repair of cisplatin-induced DNA damage in vitro (14). These clinical data, coupled with the current data on the AG and cisplatin combination in animals, support the clinical development of such a regimen in appropriate cisplatin-refractory patients. The combination of AG with cisplatin at clinically relevant doses may well have the potential to be more efficacious than cisplatin alone. It should be noted that the combination of AG and cisplatin is suggested based on the potential of AG to inhibit the function of a potentially significant cisplatin-resistance mechanism. It should be noted, however, that our current understanding of cisplatin resistance and the substantial non-MDR-1-mediated cross-resistance that accompanies primary cisplatin resistance is far from complete.

**DISCUSSION**

AG was synthesized as a water-soluble salt of aphidicolin suitable for clinical investigation. In this study the antitumor activity of AG has been established in four murine antitumor models. In each case, the activity has been demonstrated against rapidly growing i.p. tumors following i.p. administration of AG. 100 mg/kg. The combination of AG with 14 mg/kg cisplatin was less efficacious than cisplatin alone. It should be noted that the combination of AG with cisplatin at clinically relevant doses may well have the potential to be more efficacious than cisplatin alone. The combination of AG with cisplatin at 14.0 mg/kg and 4 of 8 survivors at day 230 for the combination of cisplatin (8.3 mg/kg) and AG (150 mg/kg/day for 7 days) (data not shown). The combination of AG with 14 mg/kg cisplatin was less effective (and caused 2 of 10 apparent treatment-related deaths).

**Fig. 6. Time course of DNA incorporation of [3H]thymidine in normal jejunum and i.p. B16 melanoma in B6D2F1 mice following aphidicolin, 100 mg/kg i.p.**

**Fig. 7. Use of AG and cisplatin alone and in combination in nude mice bearing the OVCAR-3 ovarian cancer cell line. AG was administered at 30 mg/kg/dose i.p. for 6 doses (every 3 h for 4 doses on day 4 postimplantation, every 3 h for 2 doses on day 5). Cisplatin doses as indicated were injected i.p. 90 min after the second AG dose.**
from complete. Even in relatively simple in vitro models of cisplatin resistance, it is apparent that resistance occurs through multiple mechanisms, including decreased drug accumulation, increased capacity for drug inactivation, alterations in the categories and perhaps the distribution of cisplatin-DNA lesions in the genome, increased repair of cisplatin-induced DNA damage in the overall genome, and increased differential repair of specific DNA sequences within the genome. We believe that these data suggest that clinical cisplatin resistance will be at least as complex. Our ongoing studies indicate that in ovarian cancer cell lines derived by combination chemotherapy-refractory patients that individual mechanisms may be the predominant contributor to resistance of individual cell lines. If these models recapitulate the clinical situation, it is clear that DNA repair may be a significant mechanism of cisplatin resistance in some individual tumors and not in others. The use of drug combinations designed to inhibit repair of cisplatin-induced DNA damage could have substantial benefit in these former patients.

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