Relationship between Patient Response in Ovarian and Breast Cancer and Platinum Drug-DNA Adduct Formation


Laboratory of Cellular Carcinogenesis and Tumor Promotion, Division of Cancer Etiology [H.S., M.C.P.]; Medicine Branch, Division of Cancer Treatment [S.G.B., E.R., C.I.A., L.P.]; and Biostatistics Branch, Division of Cancer Etiology [R.E.T.], National Cancer Institute, NIH, Bethesda, Maryland 20892

Abstract

Nucleated blood cell DNA samples from ovarian (n = 27) and breast (n = 25) cancer patients receiving either cis-diaminedichloroplatinum II (cisplatin) and/or diaminocyclobutane-carboxylatoplatinum II were examined for the presence of platinum drug bound to DNA during several cycles of therapy. Platinum-DNA adducts were quantitated by cisplatin-DNA enzyme-linked immunosorbent assay (ELISA) and atomic absorbance spectroscopy, techniques that measure either a fraction of the intrastrand cis-diamineplatinum-d(ApG) and -d(GpG) adducts (ELISA) or the total platinum bound to DNA (atomic absorbance spectroscopy), respectively. For either the complete study, or for samples obtained during the early cycles, individuals with progressive disease had severalfold lower overall cisplatin-DNA ELISA-measurable adduct levels than the individuals with more favorable clinical responses (complete response, partial response, or stable disease), who were grouped together and termed nonprogressive disease. In the case of the ovarian cancer patients, who experienced a 59% rate of complete and partial response, the correlation of high adduct values with disease response was statistically significant by the Wilcoxon rank-sum test (P = 0.028). In contrast, the breast cancer patients achieved only an 11.5% rate of complete and partial response, and the correlation of high adduct formation with disease response was not statistically significant. Levels of total DNA-bound platinum, measured by atomic absorbance spectroscopy, showed no correlation with disease response for either cancer by any analysis. The study supports previous observations demonstrating a consistent correlation between high cisplatin-DNA ELISA measurements and positive clinical outcome in ovarian cancer patients. In addition, the study provides insights into human interindividual variability in the formation of DNA adducts under controlled administration that may eventually contribute to the validation of DNA adduct-based human risk assessment.

Introduction

Cisplatin2 is a valuable antineoplastic agent currently used in chemotherapy protocols for many cancers (1, 2). Numerous clinical trials have demonstrated impressive efficacy of this drug for testicular, ovarian, lung, bladder, and head and neck cancers. Cisplatin and other cis-reacting analogues such as carboplatin form electrophilic reaction intermediates that bind covalently to DNA (3). The spectrum of DNA damage includes the major (85%) bidentate intrastrand adducts, in which the N7 positions of adjacent adenines and guanines [Pt-d(ApG)] or two adjacent guanines [Pt-d(GpG)] are covalently bound to diamineplatinum (3, 4). The remaining portion of platinum bound to DNA (<15%) is in the form of DNA monoadducts, DNA-DNA interstrand cross-links, and DNA-protein cross-links (3, 4). Furthermore, chemotherapeutically active cis-reacting analogues of cisplatin form intrastrand adducts in a fashion similar to that of cisplatin (1, 5).

Until recently, our ability to define the role of various DNA adducts in cytotoxicity and antitumor activity was limited by the lack of a sensitive method for determining platinum-induced DNA alterations. Newly developed methods that make approaches to these problems possible include immunoassays (6, 7) and AAS with Zeeman background correction (8). A polyclonal antiserum elicited against cisplatin-modified DNA has been used to develop an ELISA in which native DNA is assayed (9). This ELISA is specific for intrastrand adducts but underestimates adducts in biological sample DNA (9, 10), probably because the highly modified immunogen DNA (4.6 adducts/100 nucleotides) is conformationally different from biological samples modified to a much lower extent. The epitopes recognized by the cisplatin-DNA antiserum, which may include clusters of adducts, appear to be biologically significant since high antibody reactivity has been shown to correlate with a favorable response to therapy in ovarian and testicular cancer patients (11, 12). Quantitation of total platinum bound to DNA has been possible in biological samples using AAS. The ad-

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1 To whom requests for reprints should be addressed, at National Cancer Institute, Building 37, Room 3B25, NIH, Bethesda, MD 20892.

2 The abbreviations used are: cisplatin, cis-diaminedichloroplatinum (II); carboplatin, diaminocyclobutane-carboxylatoplatinum (II); ELISA, enzyme-linked immunosorbent assay; Pt-d(ApG), cis-diamineplatinum adduct formed on the N7 positions of two adjacent deoxyadenines; CR, complete response; PR, partial response; S, disease stabilization; PD, progressive disease; NPD, nonprogressive disease, including all individuals with a clinical response more favorable than progressive disease grouped together.

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tion of Zeeman background correction (8) has increased the sensitivity of this method to allow for the determination of platinum-DNA adducts in human DNA samples. Both methods have been used in this study since they measure different DNA damage.

The development of sufficiently sensitive assays has lead a number of laboratories to initiate extensive measurements of cisplatin-DNA adduct formation in nucleated blood cells and tissues of patients receiving chemotherapy containing platinum drugs (7, 13–16). Cisplatin-DNA adducts have been shown to accumulate in multiple tissues, including tumor and organs susceptible to platinum-drug toxicity (i.e., kidneys, peripheral nerve, and brain) (17). In addition, adducts have been measured in tissues of patients at least 15 months after the last chemotherapy, demonstrating a high degree of adduct persistence in human tissues (17, 18). Using the cisplatin-DNA ELISA, measurement of high adduct levels in nucleated blood cell DNA of 17 testicular (12) and 55 ovarian (11) cancer patients undergoing platinum drug-based chemotherapy has been shown to correlate with favorable clinical response. The data presented here expand the original observations (11, 12) with 27 additional ovarian cancer patients and 25 breast cancer patients.

Materials and Methods

Cancer Patients. The patients studied were treated by the Medicine and Clinical Pharmacology Branches of the National Cancer Institute on experimental treatment regimens approved by the National Cancer Institute’s Institutional Review Board and were not included in the studies without signed informed consent. All patients had histologically documented disease. The regimens comprised combinations of drugs including cisplatin or carboplatin or both as well as other agents. Blood samples were drawn 24 h after the last platinum-drug infusion on various cycles of chemotherapy. Disease response was assessed by physical as well as by radiographic examination. The final disease response category for each individual was determined retrospectively, based on a review of patient records, and was determined by individuals having no knowledge of the results of the DNA adduct analyses.

Disease response was categorized by standard criteria: CR was considered resolution of all detectable disease; PR comprised a decrease of >50% in tumor size; stable disease (S) was a <25% change from baseline; and PD was noted with the appearance of new lesions or a >25% increase in disease from baseline. For the purposes of the table and figures presented here, individuals with PD were compared to those with all other clinical responses, referred to collectively as nonprogressive disease. Patients were grouped in this fashion because the scarcity of CRs and PRs in the breast cancer group did not allow for a trend analysis.

Ovarian Cancer Patients. Thirty ovarian cancer patients were studied for platinum-DNA adduct formation on approved experimental regimens that included cisplatin and/or carboplatin, and 27 patients were evaluable for both DNA adducts and disease response. This group included patients receiving initial therapy and others receiving therapy for recurrent disease, some of whom had received cisplatin or carboplatin prior to the current therapy. There were 8 different protocols included in these trials. Cisplatin and carboplatin were combined in 2 protocols; cisplatin was used with or without other nonplatinum agents in 3 protocols; and carboplatin was used with or without other nonplatinum agents in 5 protocols. The cisplatin doses ranged from 40 to 100 mg/m²/cycle. The carboplatin doses were 400–800 mg/m²/cycle. Dose reductions, due to toxicity, were made in some patients after the first or second cycle. From the 27 evaluable patients 60 blood samples were assayed by cisplatin-DNA ELISA, and 46 blood samples were assayed by AAS.

Breast Cancer Patients. Thirty-four patients with recurrent breast cancer were treated with a regimen of carboplatin, 5-fluorouracil (5-FU), and leucovorin, and the clinical parameters and results of this study have been published separately (19). Carboplatin was administered at 50–100 mg/m² on days 2, 3, and 4. Leucovorin and 5-FU were given at doses of 500 and 375 mg/m², respectively, on days 1 through 5. All patients had been previously treated with surgery or chemotherapy that did not include a platinum compound. From the original 34 patients, 25 were evaluable for both DNA adducts and disease response. From these 25, 123 blood cell DNA samples were assayed by the cisplatin-DNA ELISA, and 83 were assayed by AAS.

Sample Collection and DNA Preparation. A 35–50-ml sample of blood was obtained by peripheral venipuncture or central indwelling catheter 24 h after the completion of platinum-drug treatment. All blood samples were centrifuged at 8000 rpm for 15 min at 4°C. The layered buffy coat, containing nucleated blood cells, was aspirated and frozen at −20°C until DNA was prepared. DNA isolation was performed as previously described by CsCl gradient centrifugation (20). Quantitation of DNA was by UV absorbance at 260 nm.

Cisplatin-DNA ELISA. All samples were assayed for adducts by cisplatin-DNA ELISA performed with a rabbit anti-cisplatin-DNA using 35 µg of DNA/well and having a lower limit of detection of approximately 25 amol/µg DNA, as previously described (5, 6, 9). For each assay, a standard curve was generated using calf thymus DNA modified in vitro to a level of 4.3 adducts/100 nucleotides (determined by AAS). The 50% inhibition for the standard curves averaged 12.9 ± 3.7% (mean ± SD, n = 14). DNA adduct levels for each of the biological samples were determined by comparison with the standard curve, and the units were expressed as amol of adduct/µg DNA. Each sample was assayed on 2 or 3 occasions, and on each occasion 35 µg of DNA were placed in 3 experimental and one control microtiter plate wells. Values obtained from all assays were averaged and used to determine positivity or negativity.

For samples assayed twice, a positive sample had one ELISA value of >20% inhibition and a second one ≥15% inhibition. A negative sample had one value of <15% inhibition, even if the second value was >20% inhibition. On the rare occasions when the amount of DNA was insufficient for more than one ELISA, the sample was considered negative unless the percentage inhibition was ≥25%. Each standard curve was evaluated for linearity in the lower range. The criteria outlined above were considered valid if the standard curve in question was linear down to 15% inhibition. Occasionally, when
Comprises samples obtained during the first 2 cycles or up to 600 mg/m² carboplatin for the breast cancer patients; beyond cycle 4 there were no PDs.

For all samples from each individual have been averaged to obtain a "mean" from that person, and these have been grouped by disease response and were calculated against the typical standard curve.

One-sided statistical test is not required. Unless otherwise stated in the text, P values refer to the Wilcoxon rank-sum test; 2-sided values are not necessary because an inverse correlation has never been observed.

All samples from each individual have been averaged to obtain a "mean" from that person, and these have been grouped by disease response and averaged.

Only the highest ("peak") value from each individual has been included, grouped by disease response, and averaged.

Comprises samples obtained during the first 4 cycles or up to 600 mg/m² carboplatin for the breast cancer patients.

Comprises samples obtained during the first 4 cycles or up to 600 mg/m² carboplatin for the breast cancer patients; beyond cycle 4 there were no PDs.

Table 1 Correlation between platinum-DNA adducts, measured by ELISA and AAS, with clinical outcome for ovarian and breast cancer patients

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Assay</th>
<th>Value analyzed</th>
<th>PD Mean ± Median</th>
<th>NPD Mean ± Median</th>
<th>P&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary ELISA Mean&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0 ± 5.0 (n=11)</td>
<td>0</td>
<td>60.7 ± 25.6 (n=16)</td>
<td>17.6</td>
<td>0.028</td>
</tr>
<tr>
<td>Ovary ELISA Peak&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.8 ± 11.4 (n=11)</td>
<td>0</td>
<td>172.6 ± 122.3 (n=16)</td>
<td>44.4</td>
<td>0.066</td>
</tr>
<tr>
<td>Ovary ELISA Mean&lt;sup&gt;d&lt;/sup&gt; (2 doses)</td>
<td>13.2 ± 6.8 (n=8)</td>
<td>0</td>
<td>44.4 ± 15.2 (n=12)</td>
<td>33.4</td>
<td>0.092</td>
</tr>
<tr>
<td>Breast ELISA Mean&lt;sup&gt;d&lt;/sup&gt; (4 doses)</td>
<td>51.2 ± 17.1 (n=6)</td>
<td>2.8</td>
<td>315.5 ± 151.9 (n=19)</td>
<td>30.9</td>
<td>0.24</td>
</tr>
<tr>
<td>Breast ELISA Peak (4 doses)</td>
<td>88.0 ± 59.8 (n=6)</td>
<td>8.2</td>
<td>465.2 ± 218.6 (n=19)</td>
<td>53.5</td>
<td>0.26</td>
</tr>
<tr>
<td>Ovary AAS Mean</td>
<td>30.1 ± 9.2 (n=10)</td>
<td>0</td>
<td>33.3 ± 8.3 (n=15)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Ovary AAS Mean (2 doses)</td>
<td>39.2 ± 14.0 (n=6)</td>
<td>0</td>
<td>42.4 ± 9.5 (n=11)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Breast AAS Mean (4 doses)</td>
<td>5.3 ± 2.0 (n=4)</td>
<td>0</td>
<td>10.2 ± 2.3 (n=15)</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> For cisplatin-DNA ELISA values are amol/µg DNA, and for AAS values they are fmol adduct/µg DNA; numbers are mean ± SE for the disease response group.

<sup>d</sup> One-sided P values for the Wilcoxon rank-sum test; 2-sided values are not necessary because an inverse correlation has never been observed.

<sup>b</sup> All samples from each individual have been averaged to obtain a "mean" from that person, and these have been grouped by disease response and averaged.

<sup>c</sup> Only the highest ("peak") value from each individual has been included, grouped by disease response, and averaged.

<sup>e</sup> Comprises samples obtained during the first 2 cycles or up to 200 mg/m² cisplatin and 1600 mg/m² carboplatin for the ovarian cancer patients.

<sup>f</sup> Comprises samples obtained during the first 4 cycles or up to 600 mg/m² carboplatin for the breast cancer patients; beyond cycle 4 there were no PDs.

Results

DNA Adduct Determination by ELISA and AAS in Ovarian Cancer Patients. The ovarian cancer cohort consisted of 27 individuals who were evaluated for disease response and from whom samples were obtained during 7 cycles of chemotherapy. Progressive disease was noted in 11 patients, while 8 patients achieved a PR and 8 patients a CR (CR plus PR rate = 59%). A total of 60 samples was assayed by ELISA, and 46 samples were assayed by AAS. The number and distribution of adduct determinations for the responders (CR plus PR) and the nonresponders (PD) were essentially equal.

The median and mean DNA adduct levels, obtained by ELISA and AAS for each cancer and response group, are shown in Table 1. The analyses resulting in the most significant correlation have been obtained by ELISA with samples from ovarian cancer patients given 7 cycles of chemotherapy, and the actual data points are shown in Figs. 1 and 2. The most significant correlation between adducts and disease response is presented in the first line of Table 1 (for values shown in Fig. 1A) and is based on the means of all the adduct determinations for each individual represented. ELISA values for the 2 or more samples per individual were averaged to obtain a mean value for each individual. All mean values for individuals in the PD and NPD groups were then averaged, and the differences between groups (PD versus NPD) were compared using the Wilcoxon rank-sum test. A highly significant difference was found (P = 0.028). An analysis of only the highest ("peak") ELISA value for each individual is presented in Table 1 and in Fig. 1B. The correlation for this analysis was similar (P = 0.066). Peak ELISA values were used in previous studies (11, 12) and are shown here for comparison.
Another approach was to examine only samples obtained during the first 2 cycles of chemotherapy in order to evaluate a possible predictive potential of this assay to identify individuals who might eventually respond to therapy. This included samples obtained at doses up to 200 mg/m² of cisplatin and 1600 mg/m² of carboplatin, given either separately or together, and the data are shown in Fig. 2B and Table 1. For analysis of only the first 2 doses a similar trend was observed, supporting the data in Fig. 1; however, the difference was not statistically significant (P = 0.092).

For the AAS determinations on the complete study (7 chemotherapy cycles; Table 1), 10 individuals with PD were compared to 15 with NPD, and similar values were observed in the 2 groups. For samples obtained during the first 2 cycles, the mean cisplatin-DNA ELISA adduct levels for individuals with PD were again similar to those for the NPD group.

**DNA Adduct Analyses in Breast Cancer Patients.** None of the breast cancer patients had previously received platinum drugs. Among the 25 individuals evaluable for both disease response and adducts, 6 patients had PD, 16 patients had disease stabilization, and 3 achieved either a CR or PR (CR plus PR = 11.5% for these 25 patients).

For the study as a whole, which included samples from up to 11 chemotherapy cycles, the distribution of determinations was not similar for the PD and NPD groups because there were no samples available for analysis from individuals with PD after the fourth cycle of treatment, due to patient demise or discontinuation of the unsuccessful treatment. Therefore the data presented will include DNA adduct values for samples obtained during the first 604 mg/m² of carboplatin therapy. Most patients received this quantity of drug within the first 3 or 4 cycles of treatment, and for the analysis there were 2-4 samples/patient.

For the ELISA determinations, the actual values for median and mean adduct levels were much lower in patients with PD, as compared to those with NPD (Fig. 2B; Table 1); however, neither the analysis by mean adduct level (P = 0.24) nor that using the peak adduct level (P = 0.26) was statistically significant.

For AAS analysis of the breast cancer cohort, for samples taken during the first 604 mg/m² of carboplatin administration (Table 1), 4 individuals with PD were compared to 15 with NPD, and the difference in the values was not statistically significant (P = 0.18).

**Discussion**
The data presented here demonstrate that in blood cell DNA samples from 27 ovarian cancer patients, 16 (59%) of whom achieved either a CR or a PR, high platinum-DNA adduct levels measurable by cisplatin-DNA ELISA...
correlated with the most favorable clinical outcomes ($P = 0.028$). In contrast, for 25 breast cancer patients on a protocol that gave $11\%$ CR plus PR, the trend was similar, but the results did not reach statistical significance ($P = 0.24$). These data support previous observations obtained with the cisplatin-DNA ELISA and blood cell DNA samples from ovarian and testicular cancer patients. In the ovarian cancer group examined previously (11) 1-2 samples were obtained from each of 55 individuals, and the highest (“peak”) adduct level from each person was assayed by ELISA. Disease response, classified as CR, PR, and nonresponders (including S and PD), was correlated with adduct level, and the trend was significant ($P = 0.03$). For 17 poor prognosis testicular cancer patients (12), $70\%$ experienced CR and $30\%$ experienced PR, and the $P$ value for the comparison was 0.072. Overall, the weight of evidence for the correlation between high DNA adduct levels (by ELISA) and favorable clinical outcome now rests on the analysis of 99 patients, 82 of whom had ovarian cancer.

In other studies a similar relationship has been demonstrated between disease response and DNA damage in cancer patients receiving platinum drug therapy. For example, platinum-DNA adducts were measured in blood cell DNA samples from 11 testicular cancer patients (10) using an ELISA specific for individual platinum-DNA adducts rather than the modified DNA, and even though the numbers were small the trend was identical. In another study, total platinum bound to DNA, measured by AAS, has shown a positive correlation with favorable disease response (22-24). Patients ($n = 21$) participating in a Phase I clinical trial were monitored during their first 2 cycles of therapy, and responders had significantly more adduct than nonresponders for samples taken at these times. The apparent discrepancy between these results and our AAS data (Table I) remains obscure at present but may become clear as more extensive data become available.

The cisplatin-DNA ELISA used for this study is not quantitative for intrastand Pt-d(GpG) and Pt-d(ApG) adducts, even though the primary antibody specificity is directed toward these adducts and they constitute the major fraction of platinum bound to DNA (9, 10). The cisplatin-DNA ELISA measures DNA damage, the formation of which appears to correlate with tumor remission, and the specific molecular nature of which may be related to highly modified regions within a low-modified DNA. With human tissues there is no consistency between measurement of total DNA-bound platinum by AAS and results obtained by cisplatin-DNA ELISA. As previously reported (9), when 197 human blood cell DNA samples were assayed by both cisplatin-DNA ELISA and AAS, 52 were positive in both assays, 89 were positive in AAS only, 17 were positive in ELISA only, and 39 were negative in both. A detailed analysis of the assay differences for human and animal samples is presented elsewhere (9); however, the 2 assays clearly measure different DNA damage, and there is a high degree of individual variability for the formation of cisplatin-DNA ELISA-measurable adduct in humans.

The comparison of disease response with ELISA-measurable adducts in samples obtained from ovarian cancer patients during the first 2 doses of platinum drug suggests that a systematic study on a uniform group of previously untreated ovarian cancer patients is warranted. If the current trend is validated, it may be possible to identify individuals unlikely to respond clinically by their inability to form adducts. Such information could be incorporated into an individuals overall disease management since toxicity, debilitating effects, and the expense of prolonged drug exposure might be avoided in patients who may not respond or in those with preexisting contraindications to platinum drug therapy.

DNA adduct monitoring in tissues of patients receiving DNA-damaging chemotherapeutic agents provides a unique opportunity to investigate the possibility that DNA adduct measurements may be valuable in human risk assessment. The presence of DNA adducts clearly indicates that exposure has occurred, but only in clinical populations is it possible to explore human DNA adduct processing in the context of precise dosimetry and immediate biological consequence, i.e., disease response. An evaluation of the few such studies currently available reveals a broad spectrum of interindividual variability in the formation of chemotherapy-induced DNA damage. For example, in addition to the cisplatin studies cited above (10-12, 23), studies by Hengstler et al. (25) have shown that in a population of 15 ovarian cancer patients given cisplatin and cyclophosphamide, 9 had significantly increased levels of DNA strand breaks after therapy, but 6 had no increase at all. The interindividual variability in platinum-DNA adduct formation observed in our studies is also very broad and may be due to variability in DNA repair rates or factors that alter the amount of drug reaching the target DNA. Thus, a DNA adduct-based risk assessment must necessarily recognize that many individuals will not form measurable adducts in a dose-related fashion and seek to understand the factors that contribute to interindividual variability.

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References


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S Gupta-Burt, H Shamkhani, E Reed, et al.


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