Platinum-DNA Adduct in Leukocyte DNA of a Cohort of 49 Patients with 24 Different Types of Malignancies

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ABSTRACT

Using atomic absorbance spectrometry with Zeeman background correction, we measured platinum-DNA adduct levels in leukocyte DNA of 49 patients receiving therapy consisting of only carboplatin and cisplatin. Twenty-four histological types of malignancy were included in the cohort. Peripheral blood leukocytes were collected at defined times during the first two cycles of treatment. The relationship between adduct level and disease response was highly statistically significant during cycle 1 of therapy (two-sided \( P = 0.007 \) at day 2), but statistical significance was lost during cycle 2. On all days studied, median and mean adduct levels were consistently higher in responders as compared to nonresponders (summary two-sided \( P = 0.0004 \)). These data suggest that the processes which protect cellular DNA may be common to malignant and nonmalignant rapidly dividing tissues of the same individual, regardless of the type of tumor that individual may harbor.

INTRODUCTION

Clinical resistance to platinum compounds (cisplatin, carboplatin, etc.) has traditionally been thought to be a primary function of tumor cell resistance to drug (1). The possibility that host factors may impact on the clinical response to cancer chemotherapy has been limited to concepts regarding physical properties that adhere to basic pharmacological principles, such as blood levels of drug, drug clearance, etc. Recently, studies in which antibody methodology was used in tissues from human ovarian cancer patients and testicular cancer patients demonstrate that the levels of platinum-DNA damage in peripheral blood leukocytes are highest in those individuals whose tumors shrink in response to therapy (2–5). Autopsy studies of tumor tissues and nonmalignant bone marrow show that platinum-DNA adduct levels will be similar in these two tissues from the same individual, whether measured by ELISA2 or by AAS (6–8). These observations raise the question as to whether clinical resistance to platinum drugs is truly a matter of tumor cell resistance or of pharmacogenetic properties of the host.

If clinical resistance is pharmacogenetic in origin, this may result in a very limited ability to biologically “cure” any cancer by administering only those agents whose mechanism(s) of action depend on DNA damage. Also, since the nucleotide excision repair function of cells is responsible for the repair of DNA damage caused by UV-induced pyrimidine dimers (9–12), polycyclic aromatic hydrocarbons, platinum compounds (13), and to some extent \( O^6 \)-alkylguanine lesions (14, 15), the pharmacogenetics of platinum-induced DNA damage and repair may shed light on the in vivo human biological response to a broad range of DNA-damaging agents.

We conducted a prospective blinded trial of cisplatin-DNA adduct formation in peripheral blood leukocytes of patients receiving only cisplatin and carboplatin therapy. We reported early results of this trial elsewhere (16, 17). Since then, patient accrual has doubled, resolution between three disease response groups has emerged, and the power of the statistical analysis has increased. Such a study is unique in several respects: the cohort consists of 49 patients with 24 different tumor types, the only anticancer agents received are cisplatin and carboplatin, and adduct is analyzed after the very first dose(s) of platinum-only chemotherapy.

MATERIALS AND METHODS

Patients Entered in Study. Patients were treated with an approved experimental treatment regimen, administered at the Kenneth Norris, Jr., Cancer Center of the University of Southern California Medical Center in Los Angeles. This was a phase I clinical trial of carboplatin and cisplatin to determine the maximal tolerated doses of these drugs when given in combination. All clinical data, including the assessments of disease response, were generated at the University of Southern California.

Seven dose levels were studied as described below. For the first three dose levels, patients who had not responded to prior systemic therapy were entered in the study. For the latter four dose levels, only patients who had received no previous chemotherapy were entered. Patients with a proven histological diagnosis of nonhematological malignancy were eligible for study. Eligibility criteria also included normal renal, hepatic, and neurological function, as previously described (17, 18).

Disease response categories were defined as follows. An objective partial response is a >50% reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions, lasting at least 1 month. Such patients are called RES. PD is a >25% increase in the sum of the products of the longest perpendicular diameters of all measurable lesions. SD is all of those situations that do not fit the definitions of objective response or progressive disease that last for at least the first two cycles of therapy. Patients who have stable disease or progressive disease are all NR.

Carboplatin and Cisplatin Administration to Patients. Patients studied were treated for their malignant disease by the Division of Medical Oncology of the Norris Cancer Center, University of Southern California, with an approved phase I experimental treatment protocol (17). These patients received cis-diammine(1,1-cyclobutenedicarboxylato)platinum(II) (carboplatin) on day 1 and cis-diamminedichloroplatinum(II) (cisplatin) on day 3 of 28-day cycles. Patients received doses of carboplatin ranging between 200 and 480 mg/m². Cisplatin was administered in doses of 50, 75, or 100 mg/m². Carboplatin was dissolved in sterile 5% dextrose solution and administered as an i.v. infusion over a 30-min period. Cisplatin was dissolved in normal saline and administered as a 30-min i.v. infusion after prehydration. Throughout this text the term “cycle” represents a single 28-day course of treatment.

On the morning prior to carboplatin administration (day 1), 24 h after carboplatin (day 2) and 24 h after cisplatin (day 4) administration, peripheral blood was obtained via venipuncture and centrifuged. The nucleated cells (buffy coat) were aspirated, coded, frozen at ~20°C, and transported on dry ice to the Medicine Branch of the National Cancer Institute for analysis. Each sample was then stored at ~20°C until DNA isolation. We could not obtain an adequate volume of blood from all patients on all days of the study. This resulted in the inability to compare all patients on all days of the study.
Isolation of Cellular DNA from Patient Peripheral Blood Leukocytes.
For each sample, DNA was isolated from buffy coat cells by cesium chloride density gradient centrifugation (19), as has been used in previous studies of human material (2-8). DNAs were dialyzed against double-distilled water for four exchanges over 36–48 h, and DNA content was measured by A260. The DNA concentration was adjusted as needed by lyophilization and reconstitution with double-distilled water.

The half-life of peripheral blood leukocytes is variable depending on the exact cell type. We previously reported that granulocytes (t½ = several hours) form approximately two-thirds of the WBCs in most samples and that the measured adduct level is not dependent on the proportion of long-lived or short-lived cells in the specimen (3). Adduct measured 24 h after the drug dose primarily represents DNA damage that occurred in rapidly dividing WBC precursor cells in the bone marrow compartment, which was not repaired (7).

Measurement of Elemental Platinum. DNA samples were concentrated within the range of 400 to 1000 μg/ml and assayed for total elemental platinum using a Perkin-Elmer Zeeman/3030 atomic absorption spectrometer (8). For each sample, 350 μg of DNA were analyzed and total platinum was measured against a standard curve which was generated by using aqueous serial dilutions of H2PtCl6·H2O in 10% HCl (SPEX Industries, Inc., Edison, NJ). Measurements were made in “absorbance seconds,” and platinum-DNA adduct levels were calculated as fmol Pt/μg DNA. The lower limit of detection of platinum in DNA samples was 80 pg, giving a detection limit of 400 fmol Pt/350 μg DNA.

Data Analysis. Pt-DNA adduct analyses were performed on 294 samples collected from 49 individuals. Patients making up this cohort were studied in a prospective blinded fashion. Patients received carboplatin and cisplatin therapy at one institution (University of Southern California) where buffy coats were collected and coded; DNA isolation and adduct measurements were performed on the coded samples at a different institution (National Cancer Institute). Determination of disease response was made at the University of Southern California in the absence of knowledge of the Pt-DNA adduct levels in the various patients. Comparison of adduct levels between RES and NR and between PD patients and all others (RES plus SD) on each day was based on the Wilcoxon rank sum test (20). Comparison of the trend of increasing adduct levels with increasing response status (i.e., PD < SD < RES) on each day was based on the Jonckheere-Terpstra rank test (20). A summary comparison of adduct levels between RES and all others across all 4 days was performed using the stratified Wilcoxon rank sum test (20). Two-sided P values are reported for all significance tests.

RESULTS

Shown in Fig. 1 are all of the measured adduct levels from these patients during the first two cycles of therapy. C1D1 represents the blood sample obtained on the morning prior to the first dose of therapy. C1D2 represents the blood sample taken 24 h after carboplatin administration. C1D4 and C2D4 data were obtained from blood drawn 24 h after cisplatin administration. Measured adduct levels varied between individuals by more than 10².

Fig. 1. All adduct measurements taken in leukocyte DNA during the first two cycles of therapy are shown. Patients received carboplatin on day 1 and cisplatin on day 3 of each cycle of therapy. A line connects data obtained from a single patient on different days. C1D1 and C1D2 data were obtained from blood drawn on the morning prior to carboplatin administration. C1D2 and C2D2 data were obtained from blood drawn 24 h after carboplatin administration. C1D4 and C2D4 data were obtained from blood drawn 24 h after cisplatin administration. Measured adduct levels varied between individuals by more than 10².
Table 1 Dosing sequence, drug dose levels, and adduct levels

Drug treatments were administered on days 1 (D1) and 3 (D3) of each cycle of therapy. For each dose level, median and range values of adduct measured on days 2 (D2) and 4 (D4) of cycles 1 and 2 are listed.

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Carboplatin (mg/m² body surface area)</th>
<th>Cisplatin (mg/m² body surface area)</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>75</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>75</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>320</td>
<td>50</td>
<td>4</td>
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<tr>
<td>4</td>
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<td>100</td>
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<td>5</td>
<td>400</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>480</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>480</td>
<td>75</td>
<td>11</td>
</tr>
</tbody>
</table>

Median adduct and range per dose level

<table>
<thead>
<tr>
<th>Cycle</th>
<th>D2</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>5.3 (0.0-43.4)</td>
<td>38.8 (24.4-296.9)</td>
</tr>
<tr>
<td></td>
<td>14.6 (12.3-16.9)</td>
<td>367.7 (141.6-85.9)</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>74.7 (0.0-256.4)</td>
<td>0.0 (0.0-536.1)</td>
</tr>
</tbody>
</table>

* Range of adduct values are in parentheses, which are fmol Pt/μg DNA.
* Only evaluable patient at this time.

As shown in the Fig. 1, patients who responded to therapy (A and D) form a family of curves that reflect higher adduct levels than those of patients with stable disease (B and E). The group of patients who experienced progressive disease while receiving therapy form a family of curves that cover a broad range (C and F), but most of these individuals formed adduct at levels below those of patients with responding or stable disease.

In Fig. 2, the median values for responders, stable disease, and progressive disease patients, on each day studied during the first two cycles of therapy are shown. Results of an analysis of mean values for these groups on these days follow the same pattern (data not shown). During cycle 1, responders form higher levels of adduct than stable disease patients, who form higher adduct levels than those with progressive disease. During cycle 2, the relationship between patients with progressive disease and those with stable disease is reversed.

The 24 histological types of malignancy in this cohort are listed in Table 2. In each disease response group are malignancies that are considered to be usually sensitive to platinum therapy (e.g., ovarian cancer, small cell lung cancer, and head and neck cancers) (18, 21-25) and malignancies that are considered to be usually resistant to platinum therapy (e.g., lung adenocarcinoma, colon cancer) (26-30).

As listed in Table 3, statistical analyses show that 24 h after C1D1 of platinum therapy (adduct measured on C1D2), the adduct level is related to disease response to a highly significant degree. The level of significance is reduced after the second platinum dose (C1D4) and is lost in cycle 2 of therapy. Summary stratified analyses indicated that, on all days studied, adduct levels in responders (as a group) were consistently higher than in nonresponders (two-sided P = 0.0004). Nonresponders include patients who had stable disease and those who had progressive disease.

**DISCUSSION**

Four different methods have been used to measure platinum-DNA adduct in fresh tissues from human cancer patients. The largest experience to date has been with an ELISA system which utilizes a polyclonal antiserum elicited in rabbits, developed by Poirier et al. (31, 32). Two other systems also use antibody methodology. One
PLATINUM-DNA ADDUCT MEASURED BY AAS

Table 2 Histological types of malignancy seen in this cohort

At least one responder was seen at each dose level of therapy (see Table 1 for dose levels). Numbers in parentheses, number of patients. See legend to Fig. 1 for definitions of disease response groups. Unkn, unknown.

<table>
<thead>
<tr>
<th>Disease response group</th>
<th>Median and Range of Platinum-DNA Adduct Values (fmol Pt/μg cellular DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1D2</td>
</tr>
<tr>
<td>Ovarian cancer (2)</td>
<td>42.3 (9)</td>
</tr>
<tr>
<td>Breast, adenocarcinoma</td>
<td>62.8-88.2</td>
</tr>
<tr>
<td>Esophageal, squamous cell</td>
<td>15.6 (10)</td>
</tr>
<tr>
<td>Buccal mucosa, squamous cell</td>
<td>0.0-74.4</td>
</tr>
<tr>
<td>Pleural mesothelioma (2)</td>
<td>8.8 (25)</td>
</tr>
<tr>
<td>Lung, small cell</td>
<td>0.0-45.3</td>
</tr>
<tr>
<td>Maxillary sinus, squamous</td>
<td></td>
</tr>
</tbody>
</table>

Two-sided P values

<table>
<thead>
<tr>
<th>Summary two-sided P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD vs. SD</td>
</tr>
<tr>
<td>PD vs. RES</td>
</tr>
<tr>
<td>NR vs. RES</td>
</tr>
<tr>
<td>Trend</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, numbers of patients.

The assay measures specific isolated platinum-DNA adducts (33), and the other measures adduct in intact nuclei by immunodensitometry (34). With this report, the second largest experience is now with AAS with Zeeman background correction.

The difference between responders and nonresponders in their pattern of adduct formation is that responders seem to form and/or retain higher levels of adduct in their leukocyte DNA. This pattern in platinum-DNA adduct formation has been observed in ovarian and testicular cancers and now has been observed for human malignancies regardless of the tissue of origin. We interpret this to suggest that the process operative in protecting cellular DNA from DNA-damaging agents may be similar in malignant and nonmalignant rapidly dividing tissues of the same individual. For chemotherapy to be successful, tumor tissues must have a more cytotoxic response to that therapy than tissues of the same individual. For chemotherapy to be successful, agents may be similar in malignant and nonmalignant rapidly dividing processes operative in protecting cellular DNA from DNA-damaging regardless of the tissue of origin. We interpret this to suggest that the particular cancers and now has been observed for human malignancies.

Some individuals believe that Pt-DNA adduct levels have to be related to the dose of the drug administered. If all cells were equally sensitive to Pt-DNA adduct formation, this statement would be true. Based on a large number of published in vitro studies from many different research groups, this statement clearly cannot be applied across-the-board (1, 13, 36). We believe that there is no reason to arbitrarily conclude that this statement might be true for many different individual patients, although it may be true for populations of patients.

Data presented in Table 1 suggest that the "scatter" in measured adduct levels at each dose level shows strong overlap with other dose levels. This is similar to treating a variety of tumor cell lines with the same dose of platinum. Each cell line will develop DNA damage levels that reflect innate sensitivity of that line for the development of DNA damage. Within a cell line, there will be a dose effect; but across cell lines, such an effect may or may not be observed. Our data
suggest that comparing individual patients may be more similar to comparing across cell lines than comparing doses within a cell line.

Clinically, distinctions are made between de novo resistance to therapy, as is frequently seen in lung cancer, versus "acquired resistance," as is frequently seen in ovarian cancer. Both types of "resistance" are included in this study (acquired resistance in dose levels 1, 2, and 3; de novo resistance in dose levels 4, 5, 6, and 7). Since there was at least one responder at each dose level, these data suggest that such a distinction may not be biologically meaningful. Malignancies that demonstrate de novo resistance are often those associated with prolonged periods of carcinogen exposure such as cigarette smoke, as in lung cancer (37). Carcinogen-DNA adducts have been recovered from many different normal tissues, including blood cells (38) and human placenta from mothers who smoke (39, 40). We believe that de novo tumor cell resistance to DNA-damaging chemotherapeutic agents is primarily the result of acquired resistance of that individual to DNA-damaging carcinogenic agents. For both types of compounds, the subcellular processes that protect cellular DNA are the same (1). Autopsy studies, which support this assertion, show that, following therapeutic exposures to cisplatin or carboplatin, adduct levels in bone marrow closely approximate adduct levels measured in tumor tissues (6–8).

Eight patients had measurable levels of adduct on C1D1 (the pretreatment time). All of these patients had received prior platinum therapy before beginning this protocol. Time since prior therapy ranged from 3–8 weeks. Such persistence of platinum-DNA adduct has been previously reported by our group (2, 3, 7, 16), and persistence of other types of DNA adducts have been reported by others (32, 34, 37, 38). Conversely, the absence of measurable levels of adduct after treatment in some patients tended to occur in individuals who did not respond to platinum therapy, with one exception (see Fig. 1D).

Qualitatively, our current AAS data compare favorably to our previously published data using ELISA (2–6). In a study of 55 ovarian cancer patients, observed median adduct levels by ELISA were highest in complete responders, followed by partial responders. Nonresponders had the lowest measurable adduct level. In a retrospective multivariate analysis of ovarian cancer patients who received only cisplatin or carboplatin therapy, high adduct levels were associated with a high probability of response (5). Although adduct levels measured in this study are sometimes considerably higher than those published by others, administered platinum doses in this study are also considerably higher (35, 41).

There also appears to be good agreement between the results of our current study and measured DNA damage levels associated with defined levels of cell kill in sensitive and resistant human ovarian cancer cells propagated in tissue culture (36). The levels of DNA damage measured in peripheral blood cells of patients who respond to therapy are consistent with high levels of cell kill in relatively resistant A2780/CP70 human ovarian cancer cells in vitro (36). Also, the low levels of DNA damage measured in leukocytes of patients who did not respond are consistent with good survival of A2780/CP70 cells and with substantial cell kill of only the more sensitive tumor cells we have studied, including A2780 parental cells (36) and UV repair-deficient cells (13).

In patients who receive platinum-based or platinum-only chemotherapy, the level of DNA damage measured in peripheral blood early in the treatment course is directly related to disease response. The fact that this relationship is lost with subsequent drug administrations is the subject of further investigations by our laboratory. Dabholkar et al. (42) showed that ERCC1 mRNA can be upregulated in tumor tissues and blood tissues concurrently during the course of carboplatin therapy. Therefore, the inability to repair adduct in the overall genome (36), or possibly in specific essential genes (43, 44), may be more important than the simple measurement of adduct level at a single time. We hypothesize that adduct levels are only part of the story and that the induction of DNA repair enzymes such as ERCC1 (13, 42, 45) may play an important role in determining whether a specific level of DNA damage translates into effective tumor cell kill.

REFERENCES


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