Relationship between Platinum-DNA Adducts in Leukocytes of Patients with Advanced Germ Cell Cancer and Survival

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ABSTRACT

Platinum-DNA adducts can be measured in peripheral blood leukocytes during platinum-based chemotherapy, and high adduct levels have been correlated with favorable clinical response in patients with germ cell cancer. Twenty-five patients with advanced germ cell cancer were treated with platinum-based chemotherapy regimens using the same dose and schedule of cisplatin. Platinum-DNA adducts were measured by atomic absorption spectrometry on the first and fifth days of the first cycle of cisplatin-based therapy. The patients were followed prospectively for 6–35 months (median, 26 months). Twenty-nine patients had adduct levels measured 24 h after the first dose of cisplatin. There was no difference in the mean adduct levels of those who were alive and without progression of disease compared to those who were dead or progressing (P = 0.65). Twenty-three patients had day 5 adduct levels measured. The mean day 5 adduct level in the 15 patients who were alive and without progression was 62.13 fmol/μg, compared to 153.50 fmol/μg in the patients who were dead or progressing (two-sided P = 0.02). Contrary to previous reports, these data indicate that high platinum-DNA adduct levels do not correlate with favorable outcome in patients with advanced germ cell cancer.

INTRODUCTION

Cisplatin-based chemotherapy has made curative therapy a possibility for the vast majority of patients with germ cell cancer (1). However, 20–30% of patients with advanced germ cell tumors fail to achieve a durable complete response to cisplatin-based induction chemotherapy (2). There is considerable interest in elucidating the mechanisms responsible for treatment failure in this patient population. Several investigators have measured cisplatin-DNA adducts in the peripheral blood and found high adduct level formation to correlate with favorable clinical response to cisplatin-based therapy (3–11). This relationship has been inconsistent, however, and the data have included relatively small numbers of patients with a variety of solid tumors treated in a heterogeneous fashion. For this reason, a prospective study of platinum-DNA adduct formation determined by AAS3 was conducted to explore, in a homogenous patient population (all treated with the same dose and schedule of cisplatin), whether high platinum-DNA adducts correlate with favorable 2-year outcomes.

MATERIALS AND METHODS

Patients. Men with disseminated germ cell cancer of advanced stage by the Indiana classification system were eligible (12). In brief, advanced disease under this classification includes patients with: advanced pulmonary metastases (primary mediastinal nonseminomatous germ cell tumor, >10 pulmonary metastases per lung field, or multiple pulmonary metastases >3 cm); palpable abdominal mass plus pulmonary metastases; or hepatic, osseous, or central nervous system metastases. Patients were ineligible if they were less than 18 years of age, unable to give informed consent, or expected to live less than 2 months. Patients may have received one or more prior cisplatin-based regimens. Each patient was treated with one of five different combination chemotherapy regimens in use at Indiana University Medical Center between November 1991 and September 1992 (13–16). These regimens are shown in Fig. 1. Each regimen involved the use of standard-dose cisplatin (20 mg/m²) given over a period of 15–30 min on days 1–5. Patient outcomes were determined in November 1994 by review of medical records and/or telephone follow-up with the patients’ primary physicians.

Sample Collection. Blood specimens were collected 24 h after the first dose of cisplatin as well as after the completion of hydration after the fifth dose of cisplatin during the first cycle of therapy. After obtaining informed consent, 10 cc of whole blood were obtained by peripheral venipuncture or access of an indwelling central venous catheter. DNA was isolated using the Stratagene DNA extraction kit (La Jolla, CA) and without EDTA. Four volumes of the lysing buffer [0.32 m sucrose, 10 mM Tris-HCl, 5 mM magnesium chloride, 1% Triton X-100, and 0.02% sodium azide (pH 7.6)] were added. After 2 min, the samples were centrifuged at 1500 rpm for 15 min at 4°C. The pellets were resuspended in a Tris-HCl/SDS solution. DNA was isolated from the pellets using Pronase and RNase to eliminate protein and RNA contaminants, and the DNA was stored at −20°C for future analyses.

Platinum-DNA Adducts Measured by AAS. The platinum-DNA adduct levels were measured by AAS as described

Received 9/12/95; revised 2/19/96; accepted 3/6/96.

1 Supported by the Veterans Administration Merit Review Award (to G. W. S.), National Cancer Institute Grant No. 2 R 35 CA 39844-09 (to L. H. E.), USPHS Training Grant T32 DK7519 (to M. J. F.), and a grant from the Walther Research Institute (to O. W. S.).

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3 The abbreviation used is: AAS, atomic absorption spectrometry.
**RESULTS**

Twenty-five patients agreed to participate in the study, and 23 patients were eligible for analysis. One patient was excluded because the disease extent was moderate rather than advanced. A second patient was excluded after opting to receive chemotherapy at an outside hospital. The patient characteristics and responses to therapy are shown in Table 1. Patients who were alive and without progression were followed for a median of 28 months (range 24–35 months). Patients who died or progressed had a median follow-up of 13.5 months (range 6–26 months).

The mean day 5 platination for patients who were alive and free of progression was 62.13 fmol/µg DNA (SE ± 17.36). The mean day 5 platination for patients who were dead or progressing was 153.5 fmol/µg DNA (SE ± 30.31). This difference in outcome was statistically significant using the Mann-Whitney test with a two-tailed \( P = 0.02 \). The relationship between day 5 platinum-DNA adduct levels and outcome is shown in Fig. 2. The subgroup of patients with prior cisplatin exposure was compared to the subgroup of patients with no prior exposure as shown in Fig. 3. The mean adduct level in the 9 patients with prior cisplatin exposure was 99.66 fmol/µg, whereas the mean adduct level in the 14 patients without prior cisplatin exposure was 90.21 fmol/µg.

**Table 1** Patient characteristics, chemotherapy, and outcome

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33</td>
</tr>
<tr>
<td>Median</td>
<td>17–50</td>
</tr>
<tr>
<td>Range</td>
<td>22 (96)</td>
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<tr>
<td>Primary tumor site</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Testis</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Extragonadal</td>
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<tr>
<td>Histology</td>
<td>1 (4)</td>
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<tr>
<td>Pure seminoma</td>
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</tr>
<tr>
<td>Nonseminoma</td>
<td>14 (61)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8 (35)</td>
</tr>
<tr>
<td>Outcome</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Progression-free</td>
<td>15 (65)</td>
</tr>
<tr>
<td>Dead or progressing</td>
<td>8 (35)</td>
</tr>
<tr>
<td>Prior regimens</td>
<td>4 (17)</td>
</tr>
</tbody>
</table>

**Fig. 1** Chemotherapy regimens. All cycles were given in 21-day intervals.

**Fig. 2** Relationship between DNA platination as determined by AAS and outcome of advanced germ cell tumor patients. O, patients with one or more prior therapies. □, patients with no prior cisplatin-based therapies. Horizontal lines, mean cisplatin adduct levels.
mean day 1 platination for patients who were alive and free of progression was 45.86 fmol/μg DNA (SE ± 13.20). The mean day 1 platination for patients who were dead or progressing was 53.64 fmol/μg DNA (SE ± 19.41). The Mann-Whitney test for this difference in outcome showed no statistical difference with a two-sided \( P = 0.65 \).

**DISCUSSION**

Although there are several chemotherapeutic agents that demonstrate in vivo synergy with cisplatin in advanced germ cell tumor patients, cisplatin remains the centerpiece of successful regimens. Indeed, patients resistant to cisplatin virtually all die of their disease. Thus, resistance to cisplatin-based chemotherapy remains the major barrier to cure patients with advanced germ cell tumors.

Differences in the formation of cisplatin-DNA adducts have been among the factors implicated in the expression of cisplatin resistance. There have been several studies published between 1987 and 1994 that have explored the relationship between cisplatin-DNA adduct levels and clinical response in patients with germ cell tumors and other solid tumors treated with cisplatin. These studies are summarized in Table 2. Most of these studies showed that higher platination is associated with improved clinical response or outcome (favorable result). There are, however, several characteristics of these studies that could reduce the generalizability of the results: (a) all of the prior studies included patients treated with more than one dose level of cisplatin or carboplatin; (b) the preponderance of the patients in these studies had prior therapy with cisplatin; and (c) two of the studies consisted of patients diagnosed with any one of several malignancies. More importantly, in the previously published studies, disease response is the clinical outcome measure used as opposed to progression-free survival. Progression-free survival is a more valuable outcome measure in germ cell cancer because it is common for cured patients to have residual radiographic abnormalities that simply represent necrotic fibrous tissue.

This study was designed to improve on some of the above weaknesses in the existing research regarding cisplatin-DNA adducts. AAS was chosen because it measures total platinum bound to DNA, whereas ELISA only measures about 0.2% of the total DNA-bound platinum determined by AAS (3, 7). All patients had the same disease type and stage, and all were treated with the same dose and schedule of cisplatin.

These data suggest that higher adduct formation may portend a worse prognosis. This result is similar to that shown by Motzer et al. (6) and contrary to the previous six studies reported. There are several possible explanations for these findings. For this dose and schedule of cisplatin, it is not known whether day 5 adduct formation represents the point of maximum adduct formation. Moreover, the use of leukocyte DNA hinges on the assumption that the tumor tissue resembles the surrogate tissue with respect to DNA adduct formation and clearance. In this regard, germ cell tumors may not resemble other tumor types that are less cisplatin sensitive. Another possible explanation for the findings of this study is that although AAS and ELISA are both meant to be measures of platination, the very small proportion of the total DNA-bound platinum that is determined by ELISA may have an altogether different prognostic value. This might explain why all four studies that used ELISA showed a favorable relationship between platination and clinical response. There are also clinical explanations that should be considered. Perhaps uniform cisplatin dosing and a more reliable clinical end point (progression-free survival) uncovered the true direction of the relationship between cisplatin-DNA adducts and clinical outcome. Alternatively, this relationship between platination and unfavorable outcome could be related to the presence or absence of prior cisplatin exposure. Our data did not indicate this. There was no statistically significant relationship between prior cisplatin exposure and outcome. In addition, as shown in Fig. 3, the mean platination was similar in the patients with no prior therapy compared to those who were treated with one or two prior regimens \( P = 0.90 \). However, it should be noted that the \( B \) error for this sample size was more than 0.20; thus, the possi-
bility that there may be a real difference in platination in this case cannot be excluded.

There are now data to generate conflicting hypotheses regarding the cisplatin-DNA adduct levels and outcomes in germ cell tumor patients. What is needed to clarify this relationship is a trial in poor-risk germ cell tumor patients all treated with the same regimen with a sufficient number of patients to allow analyses between those with and those without prior cisplatin exposure. It would be appropriate to include both ELISA and AAS determination of cisplatin-DNA adduct levels on the day after completion of the first cycle of cisplatin-based chemotherapy to determine the predictive values of each method in germ cell tumor patients.

ACKNOWLEDGMENTS
We thank Joanne R. Dunn for technical assistance.

REFERENCES
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