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Platinum-DNA adducts in leukocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy

(cisplatin/diamminecyclobutane-dicarboxylatoplatinum/tumor remission/human patients)

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ABSTRACT **Fifty-five ovarian cancer patients receiving platinum drug-based chemotherapy have been studied prospectively to determine the extent of formation of the bidentate intrastrand adducts of diammineplatinum covalently attached to the N^7 positions of adenosine and/or guanosine in leukocyte DNA. Data for clinical response, obtained from medical records, were then correlated with the adduct values. Patients were treated with platinum-based single-agent or combination chemotherapy containing *cis*-diamminedichloroplatinum (II) or diamminecyclobutane-dicarboxylatoplatinum on approved experimental protocols. Adduct measurements were performed by ELISA, and disease response to therapy was assessed by standard oncologic criteria. This study comprises a total of 101 blood samples obtained after intravenous *cis*-diamminedichloroplatinum (II) or diamminecyclobutane-dicarboxylatoplatinum infusion from 55 individuals, and in each case the highest (or "peak") adduct level for each patient was chosen for statistical analysis. Values for median adduct levels in patients grouped by complete response, partial response, and no response were 212, 193, and 62 amol of adduct per μg of DNA, respectively. Analysis of these data by Jonckheere's test (an extension of the Mann–Whitney test) shows that higher levels of adduct formation correlates with disease response with a two-sided *P* value of 0.030. Of eight patients on single-agent therapy whose buffy-coat samples did not have measurable adduct levels, none responded to therapy. Analysis of these data using the exact test for trend shows that the formation of adduct at a level of 160 amol/μg of DNA or greater correlates with disease response with a two-sided *P* value of 0.032. Thus in ovarian cancer patients, the formation of the intrastrand diammineplatinum adducts in leukocyte DNA is associated with favorable disease response to *cis*-diamminedichloroplatinum (II) or diamminecyclobutane-dicarboxylatoplatinum chemotherapy.**

The monitoring of macromolecular damage in tissues from humans environmentally exposed to chemical substances that interact with DNA or proteins is being actively pursued for several environmentally prevalent classes of compounds, including polycyclic aromatic hydrocarbons (1, 2), aflatoxins (3), and aromatic amines (4). Although it is anticipated that such information may lead to the determination of biologically relevant exposure levels and eventually aid in human risk assessment, validation of these studies in humans has been hampered by lack of precise exposure information (1–5). Cancer patients receiving chemotherapeutic agents that modify DNA are a group of individuals in which the administered drug dosage is precisely known and in which various biological effects can be readily monitored. We have, therefore, begun to investigate the relationship between adduct formation in leukocyte DNA and host tumor response in ovarian cancer patients receiving chemotherapy that includes either *cis*-diamminedichloroplatinum (II) (cisplatin) or diamminecyclobutane-dicarboxylatoplatinum (CBDCA). Clinical response in such patients may serve as an immediate marker for the biological response to DNA modification. Since cisplatin is a carcinogen in experimental models (6, 7), these studies may form the basis for a long-term approach to risk assessment of second malignancies. In addition they may provide information useful to the clinician in developing improved treatment protocols for the management of neoplastic disease.

Cisplatin and its analog CBDCA are potent anticancer agents that covalently bind to DNA bases, thereby disrupting normal cellular function (8–13). Cisplatin-induced DNA modifications are well characterized and include DNA interstrand cross-links (9), DNA-protein cross-links (9), DNA monoadducts (10, 12), and bidentate-intrastrand DNA adducts (10–12). A major portion of the total cisplatin bound to DNA in eukaryotic cells (11, 12) is in the form of the bidentate-intrastrand adducts of diammineplatinum covalently linked to the N^7 positions of adenosine and/or guanosine. In this laboratory these intrastrand adducts have been quantified by ELISA using a polyclonal anti-cisplatin-DNA antiserum that recognizes both adducts (refs. 14 and 15 and E.R., W. I. Sundquist, S. J. Lippard, and M.C.P., unpublished data). Although CBDCA has not been as extensively studied, preliminary reports using alkaline elution indicate that it does form interstrand cross-links similar to cisplatin (13, 16). In addition, DNA modified by CBDCA is recognized by the anti-cisplatin-DNA antiserum (G. T. Bowden, E.R., and M.C.P., unpublished data), as are other cis-reacting analogs of the parent compound (15).

Using the cisplatin-DNA ELISA we have prospectively studied 55 human ovarian cancer patients for adduct formation in leukocyte DNA. The ELISA data have been compared with disease response to therapy in the same patients and show a correlation between DNA adduct formation and a specific biological effect of the chemotherapy.

MATERIALS AND METHODS

Patient Groups. Patients studied were treated for advanced ovarian cancer (pathologic stage III or IV) by the Medicine Branch of the National Cancer Institute on approved experimental treatment protocols. Four separate groups of ovarian cancer patients are included in this study. One group, designated group HD-DDP, received cisplatin as a single

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Abbreviations: CBDCA, diamminecyclobutane-dicarboxylatoplatinum; cisplatin, *cis*-diamminedichloroplatinum (II).

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agent in the dose schedule of 40 mg/m^2/day for each of the first 5 days of a 28-day treatment cycle. These patients had been previously treated with other chemotherapy regimens that either had failed or had been initially successful but subsequently the patient had relapsed. A second treatment group was designated group CHIPS. This group received cisplatin in the dose of 20 mg/m^2/day or 30 mg/m^2/day on the first 5 days of a 56-day cycle. Other treatment received by this group included cytoxan, hexamethamelamine, the radiosensitizer misonidazole, and abdominal radiation. The third group, designated group CTX-DDP, received cisplatin in a dose of 40 mg/m^2/day and of cytoxan at 200 mg/m^2/day on the first 5 days of a 28-day treatment cycle. The fourth group, designated group CBDCA, received the cisplatin analog at the dose of 400 mg/m^2/day on each of the first 2 days of a 28-day treatment cycle. These patients, like the group HD-DDP patients, had failed to respond to previous treatments and were receiving CBDCA in a "salvage" setting as single-agent therapy. In contrast, the CHIPS and CTX-DDP groups were receiving chemotherapy for the first time.

Cisplatin and CBDCA Administration. Cisplatin was dissolved in 250 ml of 3% (wt/vol) NaCl and administered as a 1-hr i.v. infusion. When the drug dose was 20 mg/m^2/day or 30 mg/m^2/day, pretreatment and posttreatment hydration was administered at the rate of 3 liters/day continuously during the first 6 days of the treatment cycle. For the cisplatin dose of 40 mg/m^2/day, hydration was with 6 liters/day of normal saline for each of the first 6 days of the treatment cycle (17). CBDCA was administered at a dose of 400 mg/m^2/day, dissolved in 500 ml of 5% (wt/vol) dextrose solution and given as a continuous infusion over a 24-hr period; i.e., each patient received 48 hr of continuously infused drug. Throughout the text of this manuscript the term "cycle" represents a single 28-day or 56-day round of treatment, and 3–5 cycles comprise a single "course" of therapy.

Collection of Specimens, DNA Isolation, and Adduct Measurement. On the sixth day of the treatment cycle (day 4 for group CBDCA), 35–50 ml of blood was obtained via venipuncture and centrifuged at 5000 × g for 20 min. Leukocytes (the buffy coat) were then aspirated and frozen at −20°C until DNA could be isolated on cesium chloride buoyant density gradients (18). DNA was consistently prepared within 30 days of the time that the blood sample was drawn. After the DNA was isolated, it was dialyzed against water for 24–36 hr and quantitated by spectrophotometric absorbance at 260 nm. Cisplatin-DNA adducts were measured by competitive ELISA as described (19). The lower limit of sensitivity of the assay is about 25 amol of adduct per μg of DNA (19). For the purpose of data calculations, any value below this level was arbitrarily treated as if it were zero. Each DNA sample was assayed two or more times. For each assay, 35 μg of DNA was used as inhibitor in each of quadruplicate (one control, three experimental) ELISA wells. The mean value of the ELISA determinations was taken as the value for that DNA sample. Because of the large volume of blood required to generate a sufficient amount of DNA for replicate assays, one sample was drawn per treatment cycle per patient. Studies have shown that the adduct level measured in leukocyte DNA is independent of the relative proportion of the lymphocyte and nonlymphocyte components of the leukocyte count (19).

Data Analysis. We have shown that the highest portion of samples will be positive the morning following the completion of i.v. infusion of drug: i.e., the sixth day of the cycle for cisplatin (19) and the fourth day of the cycle for CBDCA (data not shown). Data from samples obtained only at these times are analyzed in this study. Disease response was determined by standard criteria that are as follows: the eradication of all evident disease represents a complete response to therapy, a >50% reduction in tumor mass is considered a partial response to therapy, and a <50% reduction in tumor mass is considered a failure to respond to therapy. Comparison was made between adduct levels observed during the course of treatment and disease response for each patient.

The results were analyzed by two different methods. Jonckheere's test, an extension of the Mann–Whitney test (20), was used to analyze adduct data by stratification on treatment group to assess the degree of nonrandomness of adduct levels between treatment groups. Using the exact test for trend in proportions, an extension of Fisher's exact test for 2 × 2 contingency tables (21), the data were analyzed to determine the degree of statistical significance attained when the data are grouped by a retrospectively chosen adduct value. Two-sided P values are reported for all significance tests.

Analysis of Autopsy Tissues of a Patient with Ovarian Cancer. A 65-year-old patient with stage III ovarian cancer expired 21 days after receiving CBDCA, cycle one, at a dose of 400 mg/m^2/day for the first 2 days of the cycle (see above). The proximate cause of death was fungal septicemia. Autopsy was performed 18 hr after the time of death. Samples were taken from 9 different tissues (see below) and were frozen immediately at −20°C without preservatives. DNA isolation and adduct determination were performed within 2 weeks of the time of death.

RESULTS

The Relationship of Adduct Level to Disease Response in Total Cohort of Patients. Adduct data for the study as a whole are shown in Fig. 1 and plotted as a function of disease-response designations. The adduct values plotted constitute the peak sample (sample with the highest adduct level) for each individual, and the median value for each disease-response group is indicated by a horizontal bar. To assess the degree of nonrandomness of the distribution of adduct levels between the three disease-response groups, an extension of the Mann–Whitney test (Jonckheere's test) was performed on the raw data shown in Fig. 1. The result of this analysis, adjusted by stratification on treatment group, is shown in Table 1. Median adduct levels in each of the disease-response groups were 212, 188, and 66 amol/μg of DNA, respectively, for complete responders, partial responders, and those who did not respond. The mean adduct levels in the respective groups were 196, 165, and 106 amol/μg of DNA. The direct correlation between actual adduct levels and disease response is statistically significant with a two-sided P value of 0.030.

To determine the significance of a specific adduct level as related to clinical disease response, these data were analyzed by the exact test for trend, seeking a specific level that would yield a P value similar to that attained by the Jonckheere analysis, noted above. Using a retrospectively chosen division of 160 amol/μg of DNA, 10 of 15 (67%) patients experiencing a complete response had high levels of adduct, compared to 9 of 17 (53%) experiencing partial response, and 5 of 23 (22%) not responding to therapy. The corresponding two-sided P value using the exact trend test adjusted by stratification on treatment group is 0.032. When individuals with adduct levels <160 amol/μg of DNA are divided into subgroups of nonadduct formers and those who formed low but measurable levels of adduct, a trend toward nonresponse is seen in both groups. However, in neither case is statistical significance reached (Table 1).

Adduct Determinations and Disease Response in Patients Receiving Single-Agent Therapy. As noted above, two of the patient groups received single-agent therapy in a "salvage" setting. One group of 13 patients received cisplatin and another group of 12 patients received CBDCA; thus a total of 25 ovarian cancer patients received single-agent therapy.

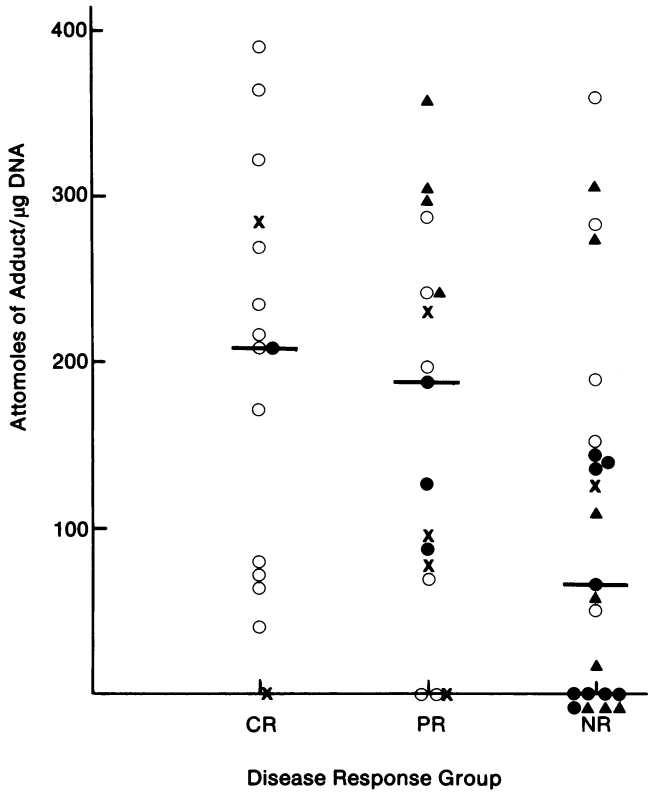

FIG. 1. Peak platinum-DNA adduct levels in leukocytes of the total cohort of 55 ovarian cancer patients treated with platinum-based chemotherapy. Patients are grouped by disease response. CR, complete response; PR, partial response; NR, no response. Treatment groups are CTX-DDP (open circles), HD-DDP (solid circles), CBDCA (solid triangles), CHIPS (×). Each data point represents the peak adduct level for a single patient. The median adduct level is shown by the heavy horizontal bar.

Adduct values and disease-response designations for these patients are shown in Fig. 2. One patient experienced a complete response, 7 patients experienced a partial response, and 17 patients did not respond to therapy. Further, most nonresponders did not form measurable levels of adducts or formed very low levels of adduct.

Statistical analysis of these data shows that median adduct values for partial responders and nonresponders are 242 and 17 amol/μg of DNA, respectively. The respective means are 230 and 74 amol/μg of DNA. Analysis of these data by Jonckheere's test shows that the relationship between adduct level and disease response is statistically significant for patients on single-agent therapy with a two-sided P value of 0.020 for the CBDCA group, 0.018 for the HD-DDP group, and 0.0015 for the two groups combined. A retrospective grouping of these samples greater than and less than 160 amol/μg of DNA showed that 75% of the patients achieving complete or partial responses (6 of 8 patients) had adduct levels >160 amol/μg of DNA in their highest sample, whereas 88% of the patients who did not respond (15 of 17 patients) had adduct levels <160 amol/μg of DNA in their highest sample. The group who formed adduct below 160 amol/μg of DNA (17 patients) can be further subdivided into two groups: 8 patients with undetectable adduct levels (<25 amol/μg of DNA) and 9 patients with levels between 25 and 160 amol/μg of DNA. Of 8 patients with platinum-DNA adduct levels >160 amol/μg of DNA, 6 patients experienced a complete or partial response, and 2 patients did not respond. In contrast, only 2 of 9 patients who formed low but measurable levels of adduct responded to therapy; and, of 8 patients who never formed measurable levels of adduct, none responded to therapy. Analyzing these data by the exact trend test, the two-sided P value is 0.0013.

Adduct Formation and Disease Response in Patients Receiving Combination Therapy. Thirty of the ovarian cancer patients studied were treated with combination chemotherapy, and data for peak adduct level and disease response for these individuals are shown in Fig. 3. Fourteen patients achieved a complete response to therapy, 10 patients achieved a partial response, and 6 patients did not respond to therapy. The median adduct levels in these disease-response groups were 213, 86, and 172 amol/μg of DNA, respectively. The respective mean adduct levels were 195, 127, and 195 amol/μg of DNA. Analysis of these data by Jonckheere's test shows a two-sided P value of 0.34, indicating that the mathematical trend toward disease response in patients on combination chemotherapy who formed higher levels of adduct is not statistically significant. Analysis of these data by the exact trend test shows a similar mathematical trend that is not significant when the adduct level of 160 amol/μg of DNA is used. Nine of 16 patients who formed ≥160 amol/μg of DNA of adduct experienced a complete response, as compared to 4 of 10 patients who formed low but measurable levels of adduct, and only 1 of 4 patients who did not form measurable levels of adduct (P = 0.54, two-sided value). Of the 4 patients on combination therapy who did not form measurable levels of platinum-DNA adduct, all responded to therapy. Two partial responders received concurrent cytoxan, and 1 partial responder and 1 complete responder received four agents (CHIPS group).

Discordance Between Adduct Level and Disease Response. Of 24 patients who formed high levels of cisplatin-DNA adduct (>160 amol/μg of DNA), 5 patients did not respond to therapy, comprising a 21% rate of discordance between

Table 1. Trend analysis of peak cisplatin-DNA adduct levels related to disease response in 55 ovarian cancer patients

	Total patients, no.	Adduct, amol/μg of DNA		Patients, no.		
		Median	Mean	>160 amol/μg of DNA	25–160 amol/μg of DNA	<25 amol/μg of DNA
Disease response						
Complete	15	212	196	10	4	1
Partial	17	188	165	9	5	3
None	23	66	106	5	10	8
P value	—	0.030*	0.030*	0.032†	NS	NS

Statistical analysis of all 55 patients studied, grouped by disease response. Analyses are performed using Jonckheere's test or the exact test for trend. NS, not statistically significant; i.e., $P > 0.05$.
*Jonckheere's test, 2-sided value.
†Exact trend test, 2-sided value.

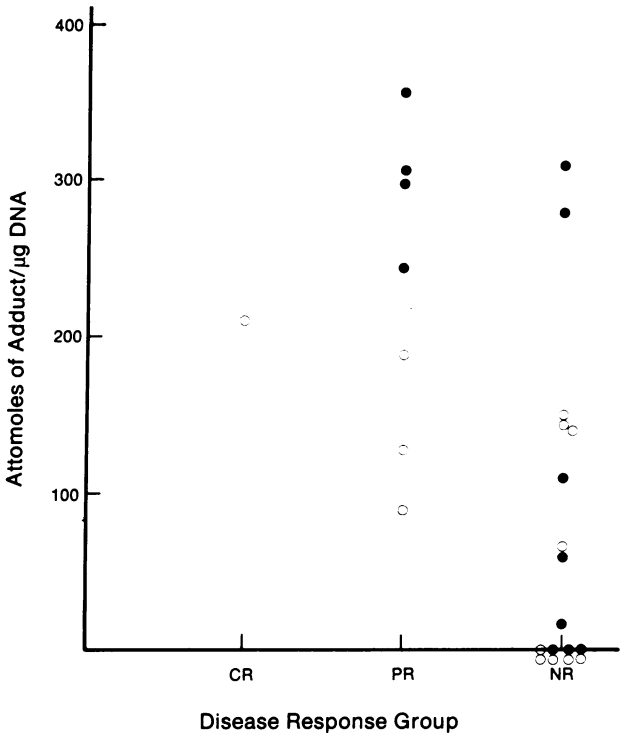

FIG. 2. Peak platinum-DNA adduct levels in leukocytes of 25 ovarian cancer patients on single-agent therapy grouped by disease response. CR, complete response; PR, partial response; NR, no response. Patient groups studied include HD-DDP (open circles) and CBDCA (solid circles). Each data point represents the peak adduct level for a single patient.

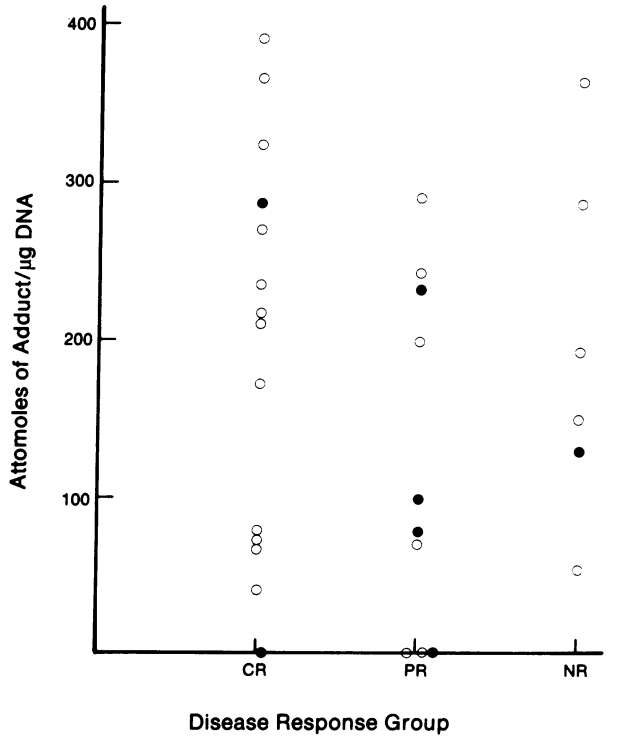

FIG. 3. Peak platinum-DNA adduct levels in leukocytes of 30 ovarian cancer patients on combination chemotherapy grouped by disease response. CR, complete response; PR, partial response; NR, no response. Patient groups studied include CTX-DDP (open circles) and CHIPS (solid circles). Each data point represents the peak adduct level for a single patient.

adduct level and disease response. These 5 patients constitute 9% of the total cohort of 55 patients. Of the 31 patients who formed low or unmeasurable levels of adduct (<160 amol/μg of DNA), 13 patients responded to therapy for a 42% discordance rate. Eleven of these patients were treated with combination therapy (groups CTX-DDP and CHIPS), and 2 patients were treated with a single agent, group HD-DDP. The 2 patients on single-agent therapy had adduct levels of 127 and 89 amol/μg of DNA, respectively. The 4 patients who had unmeasurable levels of cisplatin-DNA adduct and responded to therapy were all treated with combination drug regimens. Using the above criteria, an overall discordance rate of 33% (18 of 55 patients) was seen in this study. The discordance rate in the group receiving single-agent therapy was 17% (4 of 25 patients).

Platinum-DNA Adducts in Tissues Obtained at Autopsy from a Patient Treated with Platinum Chemotherapy. Data comparing platinum-DNA adduct levels in blood with clinical disease response imply that adduct formation in a tumor is proportional to that in leukocytes. To investigate this possibility, nine different tissues were obtained at autopsy from a 65-year-old patient with ovarian cancer who died 21 days after cycle 1 therapy with CBDCA. As listed in Table 2, seven of these tissues had measurable levels of adduct. The quantity of DNA obtained from brain white matter and bone marrow was insufficient to allow for multiple assays to make precise adduct measurements; however, the adduct levels in these tissues were >100 amol/μg of DNA. In this experiment the highest adduct levels were observed in white matter in brain, bone marrow, brain grey matter, and tumor tissue. Lower levels were seen in kidney, spleen, and lung. Adduct could not be measured in adrenal tissue or in liver. Thus it appears that platinum-DNA adducts are widespread following therapy, form in tumor tissue as well as a number of normal tissues, and persist for at least several weeks. Further studies of this sort will be required to establish a definitive relationship between the level of adducts in leukocyte DNA and tumor cell DNA. However, the high number of adducts in bone marrow and their persistence could explain the adduct accumulation observed in leukocytes (19).

DISCUSSION

In this study, the extent to which patients with advanced-stage ovarian cancer treated with platinum-based chemotherapy formed cisplatin-DNA adducts in leukocyte DNA showed a statistically positive correlation with disease response to therapy. This correlation was most striking among patients receiving single-agent cisplatin or CBDCA where the absence of adducts portended a poor clinical course. It was less striking in patients given combination therapy where some individuals in responding and nonresponding groups formed similar levels of adducts. The rate of discordance

Table 2. Platinum-DNA adduct levels measured in tissues obtained at autopsy from a patient with ovarian cancer

Tissue	Adduct level, amol/μg of DNA
Brain white matter	>100
Bone marrow	>100
Brain grey matter	122
Ovarian tumor	106
Kidney	66
Spleen	74
Lung	11
Adrenal	<10
Liver	<10

between adduct level and disease response seen in this study can be partially explained by the efficacy of nonplatinum drugs in the combination chemotherapy protocols. Of the 31 patients who formed low levels of adduct (<160 amol/μg of DNA), 13 patients responded to therapy, and 18 patients did not. Of the 13 patients who did respond, 11 patients were treated with combination therapy suggesting that other drugs in the regimen may have been responsible for the disease response observed. It is more difficult to explain patients with high levels of platinum adducts (5 out of 24 patients) who did not respond to therapy. It is possible that, in these patients, leukocyte DNA adduct levels do not correlate with disease response either because the cells can survive with more DNA adducts (22) or because more active DNA adduct removal processes (22–24) result in less long-term adduct persistence. The possibility that multiple cytotoxic agents influence the response to a single agent in combination chemotherapy is also suggested by these results. These issues may best be settled using experimental models (25) but the current results suggest the directions for study.

The presence of platinum-DNA adducts has been demonstrated here and in ref. 26 in nontumor tissues obtained at autopsy from patients who received platinum-based chemotherapy. A consistent relationship for cisplatin-DNA adduct formation in blood, other normal tissues, and tumor tissues of the same individual would imply that processes that determine the extent of platinum-DNA binding in tumor tissues may be constitutional. If such a relationship could be defined, the results might significantly alter the management of human neoplastic disease. Evidence suggests that increased levels of sulfhydryl groups (27, 28) and/or more efficient DNA repair mechanisms (22–24) may be responsible for protecting cultured cells from platinum cytotoxicity, and this may be mediated through a net reduction in platinum modification of DNA. Since the presence of sulfhydryl-containing proteins, such as glutathione, and the presence of DNA repair enzymes have been well documented in virtually all human cells including cells of hematopoietic origin (29, 30), seemingly disparate tissues in a given individual may respond similarly to a specific chemical insult. One implication of such a phenomenon for the management of human cancer would be the possibility of using autologous cultured peripheral blood lymphocytes to assess the potential for an individual to form platinum-DNA adducts and the cytotoxic response of cells to such adducts as predictors of clinical response.

The monitoring of cisplatin-DNA adducts in tissues of human cancer patients is being independently pursued by two other groups of investigators. In a report (31), total platinum bound to DNA was assayed by atomic absorption spectroscopy in tissues of five patients given either cis- or carboplatin, and values were in the range of 2–10 fmol/μg of DNA. Other investigators (32) have isolated cisplatin DNA adducts on HPLC and used a series of immunoassays to determine each adduct individually. Adducts from several patients ranged between 0.2 and 9.0 fmol/μg of DNA, with three patients giving the very high values and the others falling more in the range of those reported here—that is 0.025–0.4 fmol/μg of DNA. Differences in absolute adduct levels may be related to assay methods, dosing schedules, and/or other factors.

In addition to the implications for management of human neoplastic disease already discussed, these studies are useful in the assessment of biologically effective chemical dosages in human exposure. In human studies designed to monitor DNA-adduct formation resulting from environmental and occupational exposure (33), information has been lacking on the precise exposure dose, and it has often been difficult to identify unexposed controls. Both of these problems are overcome in the present study, since administered cisplatin doses are precisely known and unexposed controls are readily available. Although cisplatin is not a proven human carcinogen, it is carcinogenic in rodents (6, 7). Of concern is the possible induction of second malignancies in long-term survivors of cancer who have been successfully treated for their malignancy (34–36). The methodology employed in this study, therefore, is likely to have practical implications for both cancer treatment and cancer epidemiology.

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