Platinum Drug–DNA Interactions in Human Tissues Measured by Cisplatin–DNA Enzyme-linked Immunosorbent Assay and Atomic Absorbance Spectroscopy

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Studies of platinum drug–DNA adduct formation in tissues of cancer patients have involved both atomic absorbance spectroscopy (AAS), which measures total DNA-bound platinum, and anti-cisplatin–DNA enzyme-linked immunosorbent assay (ELISA), which detects a fraction of the AAS-measurable adduct. These studies were designed to explore mechanisms of drug–DNA interactions, to make correlations with clinical outcome, and possibly to validate DNA adduct measurements for use in occupational and environmental biomonitoring. The results, determined by both ELISA and AAS, demonstrate that cisplatin and its analog carboplatin bind to DNA in many human organs, including kidney, brain, peripheral nerve, and bone marrow, which are sites for drug toxicity. Platinum was also observed bound to ovarian tumor DNA. The adducts were highly persistent, being measurable in tissues obtained at autopsy up to 15 months after the last administration of platinum chemotherapy. A comparison of blood cell DNA adduct levels, determined by ELISA, and the clinical response of 139 patients with ovarian, testicular, colon, or breast cancer demonstrated a strong correlation between failure to form DNA adducts and failure of therapy. Conversely, patients who formed high levels of DNA adduct were most likely to respond favorably. A similar correlation was not observed for adducts determined by AAS; that is, the average total DNA-bound platinum levels were the same for patients who did not respond to therapy and for patients who had any kind of response. Thus, in this study, human blood cell DNA adducts measured by ELISA correlate with tumor remission, while those measured by AAS do not.

Introduction

A number of effective anticancer drugs are known to damage DNA, and their selective cytotoxicities for neoplastic cells are presumably related to an increased sensitivity of tumor cells as compared to normal cells. Newly developed assays have made it possible to monitor the binding of drugs such as 8-methoxypsoralen (*1*), procarbazine (*2*), mitomycin C (*3*), and cisplatin (*cis*-diamminedichloroplatinum II) (*4*) bound to DNA in tissues of cancer patients receiving chemotherapy. While such studies are directed toward determining the biologically effec-

tive dose of drug to target tissue and correlating tumor response, they also serve to validate assays for DNA adduct formation in humans, which are being used for occupational and environmental biomonitoring (*5*).

Cisplatin binds to DNA in a chemically stable fashion to produce, primarily, intrastrand bidentate diammineplatinum adducts by chloride displacement at the N7 positions of adjacent guanines and adenine–guanine (5'–3') dinucleotides (*6,7*). This compound, and its *cis*-reacting analogs such as carboplatin (diamminecyclobutane-dicarboxylatoplatinum II), are among the most effective drugs currently available for the treatment of testicular, ovarian, lung, and other cancers (*8–10*). In recent years a number of highly sensitive enzyme-linked immunosorbent assays (ELISAs) have been developed (*4,6*) and used to measure binding of platinum drugs to DNA in tissues of cancer patients (*11,12*). In addition, atomic absorbance spectroscopy (AAS), with the addition of Zeeman background correction, has become sufficiently sensitive to measure total platinum bound to DNA in human samples (*13*).

This paper focuses on platinum drug–DNA interactions in human tissues. It includes: a comparison of ELISA and AAS for the determination of cisplatin–DNA adducts in humans; a demonstration of widespread adduct formation and long-term adduct persistence in human tissues; and a demonstration of a positive correlation between adduct formation measured by cisplatin–DNA ELISA and tumor remission.

Materials and Methods

Clinical Studies

Eight cancer patients studied at autopsy had been treated for ovarian or breast cancer or lymphoma on approved experimental protocols at the Medicine Branch of the National Cancer Institute (14,15). Each patient had received one or more cycles of chemotherapy containing either cisplatin or carboplatin and had died 1 day to 15 months since their most recent platinum therapy. Autopsy was performed within 24 hr of the time of death, and samples were taken of bone marrow, lymph nodes, spleen, kidney, liver, peripheral nerve, and brain. From ovarian cancer patients, samples of the ovarian tumor were obtained. The medical records of the eight individuals were reviewed to obtain the following information: age, gender, cause of death, type and duration of response to the most recent platinum treatment regimen, time since most recent platinum dose, total cumulative drug dose, and number of courses of therapy prior to death.

The patients from whom blood was obtained had also been treated on approved experimental treatment protocols at the National Cancer Institute for ovarian (14,16), breast (16), colon (16), or testicular (17) cancers and had received either cisplatin or carboplatin. Multiple protocols, most of which were combination therapies, had been used. Disease response criteria, obtained from the medical records of each patient, were categorized as follows: progressive disease is a > 25% increase in tumor size; stable disease is a < 25% change from baseline, either increase or decrease; a minor response is a 25–50% decrease in tumor size; a partial response is a > 50% decrease in tumor size; and a complete response is resolution of all clinically detectable disease. No response is a generic term which includes progressive and stable disease and minor response. Blood was drawn 24 hr after the most recent platinum drug infusion.

Isolation of DNA

All cell pellets and tissues were frozen at $-20°C$ for 1–2 months before DNA preparation. DNA was isolated on CsCl buoyant density gradients, as previously described (18). The salt was removed by dialysis and the DNA content determined by ultraviolet absorbance at 260 nm.

Cisplatin–DNA ELISA

The cisplatin–DNA ELISA was performed with a rabbit anti-cisplatin DNA using 35 μg of DNA per well, as previously described (4,19). For each assay, a standard curve was generated using calf thymus DNA modified in vitro to a level of 4.3 adducts per 100 nucleotides (determined by AAS). The 50% inhibition for the standard curves averaged 12.9 ± 3.7 fmol/μg DNA (mean ± SD, n = 14). DNA adduct levels were determined for each biological sample by comparison with the standard curve and expressed as attomoles of adduct per microgram DNA.

Atomic Absorbance Spectroscopy

Total platinum–DNA binding was assessed by AAS with Zeeman background correction, as previously described (13), using a Perkin-Elmer Zeeman/3030 atomic absorption spectrophotometer. Samples of 200 μg DNA were used for each AAS measurement, and quantification was done by comparison with a standard curve. Units used are femtomoles of adduct per microgram DNA.

Results

Comparison of Cisplatin–DNA ELISA and AAS for Analysis of DNA Adducts in Human Samples

Whereas AAS measures total platinum bound to DNA, the cisplatin–DNA ELISA significantly underestimates the total amount of intrastrand DNA adducts. This observation was first made in samples from rats (19,20). Although ELISA measured only 0.2% of the total DNA-bound platinum determined by AAS, a linear correlation was observed between the two assays in kidneys of rats exposed to increasing doses of the drug. A similar consistency was not found in human samples (16,19). In a recent study in which 197 blood-cell DNA samples were assayed by both ELISA and AAS, 39 samples gave negative results in both assays, 17 gave positive results in ELISA only, 89 gave positive results in AAS only, and 52 gave positive results in both assays. A comparison of the values obtained by ELISA and AAS for each of the positive samples is shown in Figure 1. The fraction of total platinum determined in these 52 samples by ELISA varied between 0.2 and 33% of that observed by AAS, with almost half of the samples falling in the range of 2–33%. A similar relationship appears to hold for human tissue obtained at autopsy; nine samples gave positive results in both assays and showed similar inter-assay discrepancies (compare Tables 1 and 2).

Platinum–DNA Adducts in Human Tissues Obtained at Autopsy

Although blood-cell DNA samples are readily obtainable for analyses of DNA adducts, they provide no direct information about the amount of DNA damage present in the target tissue (tumor) or in other organs that may be sites for toxicity. In addition, such studies tell us little about platinum–DNA adduct persistence in specific tissues. Tables 1 and 2 present data on the tissues of eight cancer patients who had received their last dose of cisplatin or carboplatin between one day and 15 months prior to death and autopsy (15). The patients had either lymphoma (patient 1), breast cancer (patient 2), or ovarian cancer (patients 3–8). The tissues studied included ovarian tumor, bone marrow, lymph node, spleen, kidney, liver, peripheral nerve, and brain. Time since last platinum chemotherapy was 7–15 months for patients 5–8, 2 and 4 months for patients 1 and 4, respectively, and less than 1 month for patients 2 and 3. It is evident from Tables 1 and 2 that widespread distribution of platinum–DNA adducts occurs and that the quantities of cisplatin–DNA ELISA-measurable adduct levels in tumor are similar to those seen in other tissues.
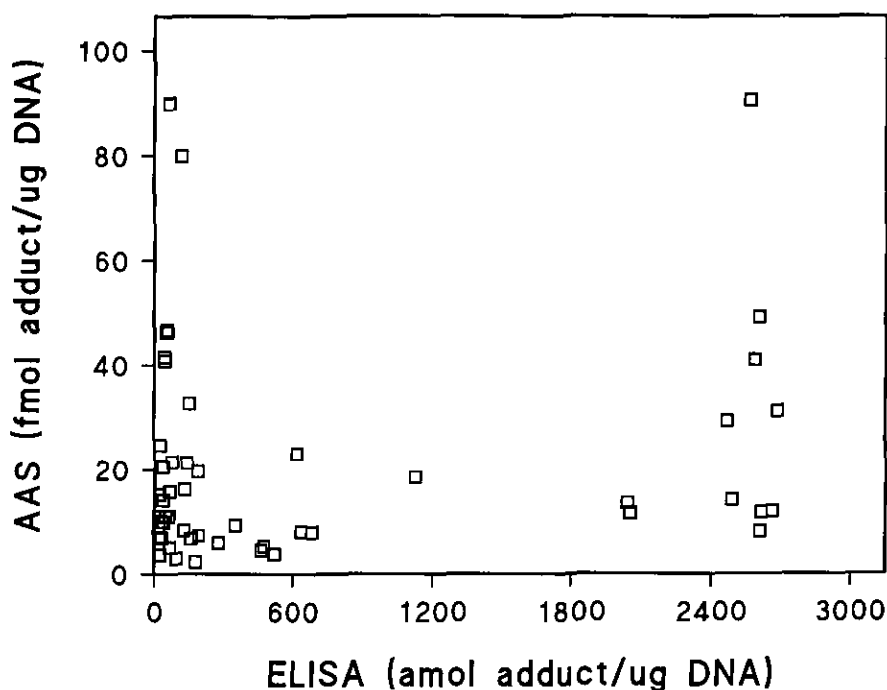
FIGURE 1.   Comparison of values obtained by cisplatin–DNA enzyme-linked immunosorbent assay (ELISA) and atomic absorbance spectroscopy (AAS) for the same 52 human blood-cell DNA samples.

Table 1. Platinum–DNA adducts (amole/μg DNA) determined by enzyme-linked immunosorbent assay (ELISA).[a]

Tissue	Patient number							
	1	2	3	4	5	6	7	8
Tumor	—[b]	—	106	58	—	176	73	—
Bone marrow	—	ND[c]	100	77	—	141	—	—
Lymph node	ND	143	—	ND	—	—	—	—
Spleen	88	343	74	—	141	—	283	113
Kidney	—	511	66	50	—	315	122	184
Liver	—	457	10	45	211	342	96	78
Peripheral nerve	—	ND	—	ND	—	62	ND	315
Brain (gray)	—	143	122	62	—	—	—	833
Brain (white)	—	306	100	112	—	—	—	456

[a]Mean of two ELISAs performed on separate occasions; each assay included triplicate experimental wells and one control well per sample.

[b]Dash indicates no sample assayed.

[c]ND, levels below 8 amole/μg DNA, the lower limit of sensitivity.

Table 2. Platinum–DNA adducts (fmole/μg DNA) determined by atomic absorbance spectroscopy.

Tissue	Patient number					
	1	2	4	5	6	7
Tumor	—[a]	—	ND[b]	—	ND	11
Bone marrow	4	ND	ND	—	—	14
Lymph node	0	28	14	—	—	—
Spleen	14	ND	ND	59	—	53
Kidney	—	41	6	—	204	38
Liver	—	23	29	11	90	198
Peripheral nerve	ND	ND	—	4	19	
Brain (gray)	—	ND	22	—	—	—
Brain (white)	—	ND	ND	—	—	—

[a]Dash indicates no sample assayed.

[b]ND, levels below 3 fmole/μg DNA, the lower limit of sensitivity.

Comparison between Platinum–DNA Adduct Formation Response in Human Blood Cell DNA and Disease

Previous determinations of cisplatin–DNA ELISA-measurable adducts in blood-cell DNA from ovarian (14) and testicular (17) cancer patients demonstrated a positive correlation between adducts and favorable clinical outcome (Table 3, groups 1 and 3). In these investigations, one or two samples were obtained from each patient, and the highest or "peak" adduct value was considered for comparison with disease response. Clinical response was categorized as presented in Methods. In a group of 24 patients (Table 4) who received single-agent platinum therapy, the correlation between adduct level and disease response was very highly significant ($p = 0.006$) (21). Overall, patients with the best clinical response had the highest adduct levels. The studies are, however, limited to two types of cancer and one assay procedure.

Recently, these observations were repeated in ovarian cancer patients and extended to breast and colon cancer patients, as shown in Table 4 (16). The new study has the added advantage that measurements of adducts by ELISA were compared to those by AAS for the same samples. The study comprised a total of 286 blood samples obtained from 67 patients, with 1–2 samples obtained from 36% of patients, 3–9 samples from 55% of patients and > 9 samples from 9% of patients. Multiple samples were taken from each individual at intervals during treatment; because of substantial variation in the numbers of samples per person, a mean adduct value was calculated for each patient. The data for patients grouped by disease response are presented in Table 3 (groups 2, 4, and 5). The ovarian cancer patients again showed a statistically significant correlation between adduct formation

Table 3. Summary of comparisons between cisplatin–DNA adducts measured by enzyme-linked immunosorbent assay and disease response for 139 cancer patients.

Group no.	Cancer	No. of patients	Type of Pt therapy[a]	Prior therapy	% Complete and partial responders	Mean (amole/μg DNA) Nonresponder[b]	Responder[c]	p
1	Ovarian[b]	24[d]	SA	(+)	33	79	227	0.006
1	Ovarian[e]	55	SA + C	(+)	58	106	179	0.03
2	Ovarian[f]	27	C	(+)	59	9	61	0.028
3	Testicular[e,g]	17	C	(−)	70 (complete)	83	162	0.072
4	Breast[f]	26	C	(+)	11	51	248	0.047
5	Colon[f]	14	C	(+)	21 (partial)	140	223	0.24

[a]SA, single agent; C, combination.

[b]The nonresponders in groups 2, 4, and 5 had progressive disease.

[c]Responders include complete and partial responders. For the breast and colon cancer patients, this group also includes individuals with stable disease, since there were so few complete and partial responders.

[d]A subgroup of the 55 patients shown in the next group.

[e]Values are peak or highest adduct level from each individual.

[f]Values are mean of several adduct levels, determined from DNA samples taken at different times from the same individual.

[g]There were no nonresponders, so complete responders were compared to partial responders.

Table 4. Platinum–DNA adducts in blood-cell DNA from 67 cancer patients.

Group no.	Tumor	No. of patients	Method of measurement[a] ELISA	AAS
2	Ovary	27	60	46
4	Breast	26	131	83
5	Colon	14	95	68
Total		67	286	197

[a]ELISA, enzyme-linked immunosorbent assay; AAS, atomic absorbance spectroscopy.

and positive clinical outcome. The breast and colon cancer groups contained fewer responders, but the trend for differences in adduct levels between responders and nonresponders were consistent with those observed for the ovarian and testicular cancer patients.

In this study, it was possible to measure total platinum bound to DNA by AAS for 197 of the original 286 blood-cell DNA samples. Figure 2 shows a comparison of the mean ELISA and AAS values for patients with progressive disease, compared to patients with either stable disease, partial response, or complete response, grouped together. Analysis by ELISA showed a statistically significant difference between the two groups, but analysis by AAS showed similar adduct levels for both groups. Therefore, in this study, when averaged adduct levels were used, high levels of adduct measured by AAS did not correlate with tumor remission.

Discussion

In order to establish a cisplatin–DNA ELISA, DNA modified to 4% with cisplatin was used to elicit antisera specific for the intrastrand bidentate N7-d(GpG)- and N7-d(ApG)-diammineplatinum adducts. These adducts make up a major portion of platinum bound to DNA; however, the ELISA developed with this antiserum provides an underestimate of the quantity of intrastrand adduct present, presumably because biological samples (1 adduct in 10^7 nucleotides) have many fewer adducts in proximity than the original immunogen (1 adduct in 25 nucleotides). It is possible that the antiserum has highest affinity for clusters of adducts and that the binding affinity is considerably reduced for a single adduct. It is also possible that it is clusters of adducts that are primarily recognized in biological samples. In any case,
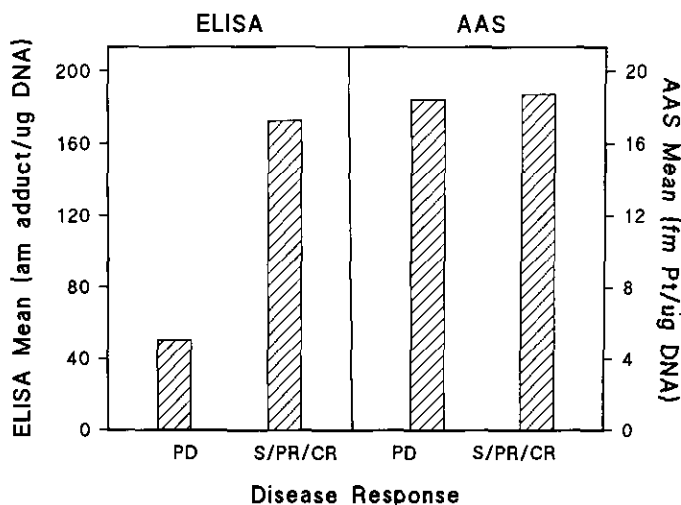

FIGURE 2. Comparison between average platinum–DNA adduct levels measured by cisplatin–DNA enzyme-linked immunosorbent assay (ELISA) or atomic absorbance spectroscopy (AAS), with disease response for 67 patients with either ovarian, breast, or colon cancer. Individuals with progressive disease (PD; ELISA, n = 23; AAS, n = 20) are compared to those with stable disease (S), partial response (PR) or complete response (CR), grouped together (ELISA, n = 44; AAS, n =42).

the underestimation obtained with the cisplatin–DNA ELISA can be circumvented by using AAS or by performing ELISA with an antiserum elicited against individual intrastrand adducts rather than modified DNA (20,22). The latter assay has the disadvantage that samples must be column chromatographed prior to analysis by ELISA, thereby increasing cost and reducing efficiency.

A comparison of results obtained using AAS and the cisplatin–DNA ELISA to analyze the same patient blood-cell DNA samples demonstrated no consistent correlation between the two assays (19). This finding was in contrast to that with rat samples, for which the two assays exhibited a constant relationship (19). Human DNA samples have, in general, much more ELISA-recognizable material than rat kidney DNA samples or mouse skin samples. In addition, a much broader range of interindividual variability in cisplatin–DNA ELISA-measurable adducts has been observed in human cancer patients than in inbred rats.

The data on platinum–DNA adducts in tissues obtained at autopsy clearly demonstrate widespread distribution and long-term persistence of both ELISA- and AAS-measurable adducts. The results show that adducts form in tissues such as kidney, peripheral nerve, bone marrow and brain, which are sites of drug toxicity, as well as in tumor tissue. The high degree of persistence of these adducts (14–15 months for patients 8 and 6, respectively) suggests that failure to repair platinum–DNA damage may contribute to the chemotherapeutic efficacy of this class of drug.

The cisplatin–DNA ELISA has been used extensively to assay human blood-cell and tissue DNA, since much of this work was performed prior to our knowledge of the underestimation. Table 3 shows the results of two major studies, involving 139 patients, which demonstrate a unique correlation between ELISA-measurable adducts and positive clinical outcome. In general, the absence of cisplatin–DNA ELISA-detectable adducts appears to be associated with poor disease response, and patients who have the highest adduct levels are the most likely to achieve remission. The consistency of the correlation (Table 3) strengthens the evidence for a direct association between high platinum–DNA adduct levels and positive disease response.

In contrast, the results of AAS determinations on the same samples show no similar correlation. It should be noted that Reed et al. (23) demonstrated a positive correlation between disease response and DNA adducts measured by AAS. Their study comprised 21 patients with 15 different tumor types who were participating in a phase-1 trial. The ELISA for Pt-d(GpG) intrastrand adducts, established with an adduct immunogen rather than a modified-DNA immunogen, has also been used to investigate the adduct–disease response correlation (24,25); preliminary results with 11 samples suggest that this assay may also show a correlation. Several laboratories are just beginning to examine this correlation; the most extensive data currently available were obtained with the cisplatin–DNA ELISA.

Conclusions

One goal of disease–response correlations is presumably to predict response in a particular patient. Given human interindividual variability and the fact that the most effective regimens contain a combination of drugs, however, it is not clear that this assay will become a useful tool for predicting response. It will nevertheless allow further investigation of factors that modulate platinum–DNA adduct formation and continue to enhance our understanding of platinum drug–DNA interactions.

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