

Influence of Cellular Factors and Pharmacokinetics on the Formation of Platinum-DNA Adducts in Leukocytes of Children Receiving Cisplatin Therapy¹

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ABSTRACT

The formation of platinum (Pt)-DNA adducts is thought to be crucial to the antitumor activity of cisplatin, and relationships between adduct formation in peripheral blood leukocytes (PBLs) and response to cisplatin therapy have been reported. The current study directly tests, for the first time, whether pharmacokinetic or other factors predominantly determine the drug-target interaction of cisplatin in a pediatric patient population.

Cisplatin pharmacokinetics and Pt-DNA adduct formation in PBLs were determined in 10 children in parallel with measurement of adduct levels after incubation of pretreatment blood samples with cisplatin *in vitro*. Total and unbound plasma Pt concentrations were determined by atomic absorption spectrophotometry and adduct measurements performed by competitive ELISA.

Pt-DNA adduct levels determined after cisplatin treatment showed considerable interindividual variation (peak levels at 24 h ranged from 0.15 to 1.31 nmol/g DNA) and correlated strongly with adduct levels determined after incubation of pretreatment whole blood with cisplatin ($r = 0.92$; $P = 0.0002$). No significant correlation was observed between *in vivo* adduct formation and either unbound or total cisplatin plasma concentrations ($r = 0.14$ and 0.18 , respectively). A correlation was also observed between the degree of myelosuppression, as determined by WBC nadirs measured over a 14-day period after cisplatin treatment, and the extent of adduct formation, with greater WBC

toxicity observed in patients with higher levels of Pt-DNA adducts ($P = 0.010$).

These preliminary results provide evidence that interpatient variation in formation of Pt-DNA adducts in PBLs of children is determined by host-specific factors other than cisplatin pharmacokinetics. These results imply that analysis of adducts in PBLs after incubation of pretreatment blood samples with cisplatin may be used to predict *in vivo* adduct levels, leukopenia, and, potentially, response to cisplatin therapy.

INTRODUCTION

The antineoplastic agent cisplatin is widely used for the treatment of various types of cancer in adults and is currently an essential component in many clinical pediatric protocols for the treatment of osteogenic sarcoma, neuroblastoma, and central nervous system tumors (1–3). The clinical application of cisplatin is limited by its severe side effects including nephrotoxicity, neurotoxicity, and the induction of nausea and vomiting (4, 5) and by the development of tumor resistance to this agent (6). Because cisplatin is commonly used for the treatment of tumors with relatively low long-term cure rates, in addition to the serious side effects and potential resistance associated with its use, the identification of a marker for predicting response and/or toxicity to cisplatin would be an invaluable tool. Individualization of cisplatin therapy could potentially lead to the identification of patients who are unlikely to benefit from cisplatin treatment as well as those likely to suffer more serious side effects.

The activity and toxicity of cisplatin, in common with other anticancer drugs, is likely to be influenced, to a greater or lesser extent, by both pharmacokinetic and pharmacodynamic factors. For example, the antitumor and toxic effects of the cisplatin analogue carboplatin have been related to its pharmacokinetics in several studies in both children and adults (7–10). This has led to the concept of basing carboplatin dosing on a target AUC³ and the development and validation of dosing formulas based on renal function in adult and pediatric patient populations (11, 12). This individualization of dosing results in more consistent exposure to carboplatin than surface area-based drug administration (13) and allows carboplatin exposure to be optimized in individual patients (14). However, such an approach has not been taken forward with cisplatin, for which dosing in children is commonly based on body surface area or body weight. This

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³ The abbreviations used are: AUC, area under the plasma concentration-time curve; Pt, platinum; AAS, atomic absorption spectrophotometry; PBL, peripheral blood leukocyte; AUA, area under the DNA adduct-time curve; C_{max}, maximum concentration.

Table 1 Summary of clinical studies investigating cisplatin-DNA adduct interactions in PBLs

Author (reference)	Patient population	Tumor type	Assay method	Correlations investigated		
				Adducts/response	Adducts/PK ^a	Adducts <i>in vitro/in vivo</i>
Reed <i>et al.</i> (18)	Adult	Testis/ovary	ELISA	+ ve	ND	ND
Reed <i>et al.</i> (19)	Adult	Ovary	ELISA	+ ve	ND	ND
Fichtinger-Schepman <i>et al.</i> (21)	Adult	Various	ELISA	+ ve	ND	+ ve
Parker <i>et al.</i> (20)	Adult	Various	AAS	+ ve	ND	ND
Gupta-Burt <i>et al.</i> (27)	Adult	Ovary/breast	ELISA/AAS	+/- ve	ND	ND
Motzer <i>et al.</i> (26)	Adult	Testis	ELISA/AAS	- ve	ND	ND
Schellens <i>et al.</i> (16)	Adult	Various	AAS	+ ve	+ ve	ND
Bonetti <i>et al.</i> (23)	Adult	Various	ICPMS	- ve	ND	- ve
Peng <i>et al.</i> (24)	Pediatric	Various	ELISA	ND	- ve	ND

PK, pharmacokinetics; ICPMS, inductively coupled plasma mass spectroscopy; ND, not determined.

leads to large interpatient variation in actual exposure to the active drug, as determined by the free cisplatin AUC (15, 16).

The Pt drugs are thought to exert their antitumor effects by reacting with DNA to form Pt-DNA intra- and interstrand cross-links or adducts (17, 18). Numerous studies over the past decade have investigated the factors involved in determining cisplatin-DNA adduct interactions and their potential clinical relevance; these studies are summarized in Table 1. The extent of Pt-DNA adduct formation in PBLs has been correlated with clinical response in several studies, suggesting that adduct formation in normal cells may parallel that in tumor tissue (16, 18–20). Additional studies have suggested possible correlations between DNA adduct levels formed in PBLs after cisplatin treatment and adduct formation in PBLs from the same patients incubated with cisplatin *in vitro* (21, 22), although contradictory data have since been published (23). Similarly, a relationship between the pharmacokinetics of cisplatin and Pt-DNA adduct formation has been reported in an adult study (16), but a comparable relationship was not observed in a pediatric patient population (24). The limitation of many of these studies is that pharmacokinetic and pharmacodynamic parameters were investigated separately, *i.e.*, the potential influences of these two key parameters on Pt-DNA adduct formation were not compared in the same study. Differences in the results published to date may reflect differences in assay methods, patient populations, or experimental differences in sample handling.

The current study was designed to investigate the influence of cisplatin pharmacokinetics on Pt-DNA adduct formation in pediatric patients. In parallel, the potential correlation between adducts formed *in vitro* after incubation of pretreatment blood with cisplatin and adduct levels measured *in vivo* was examined. Results from this study will determine for the first time whether pharmacokinetic or cellular factors predominantly influence the drug-target interaction of cisplatin in a pediatric patient population.

MATERIALS AND METHODS

Patients and Treatment Details. Ten children and adolescents receiving cisplatin treatment at the Children's Cancer Unit of the Royal Victoria Infirmary and Newcastle General Hospital (Newcastle upon Tyne United Hospitals Trust, Newcastle upon Tyne, United Kingdom) were entered into the study.

Patients were studied between January 1999 and June 2000. The study protocol was approved by the Newcastle Health Authority and the University of Newcastle upon Tyne Joint Ethics Committee, and written informed consent was obtained for all of the patients. Patient characteristics including age, sex, body weight, surface area, diagnosis, chemotherapy regimen, and cisplatin dose are given in Table 2.

Cisplatin (50–100 mg/m²) was administered as a 24-h infusion in normal saline with pre- and posthydration. Treatment was given as part of the standard chemotherapy regimen that each patient was currently receiving (Table 2), and dosing was based on body surface area in all of the cases.

Blood Sampling. Blood samples (3 ml) for pharmacokinetic analysis were obtained from a central line before cisplatin infusion, at 1, 2, and 6 h during infusion, at the end of infusion, and 24 h after the end of infusion. Plasma was separated from whole blood samples by centrifugation (1200 × *g*, 4°C, and 10 min), and 1 ml was then removed and placed in an Amicon Centrifree micropartition unit with a *M_r* 30,000 cutoff (Millipore, Edinburgh, United Kingdom). This plasma sample was centrifuged (1500 × *g*, 4°C, and 15 min) to obtain plasma ultrafiltrate for determination of free cisplatin levels, and both plasma and ultrafiltrate samples were stored at –20°C before analysis.

Additional blood samples (10 ml) were collected into lithium heparin tubes at pretreatment, at 6 h during infusion, at the end of infusion, and 24 h after the end of infusion for measurement of Pt-DNA adduct levels. Immediately after collection, blood samples were layered onto Polymorphprep (Nycomed Pharma AS, Oslo, Norway) and centrifuged (500 × *g*, 20°C, and 35 min). PBLs were collected, washed twice with PBS, and the cell pellet stored at –20°C before analysis.

In Vitro Cisplatin Incubations. Blood samples (10 ml) taken from children before cisplatin treatment or from healthy volunteers were collected in lithium heparin tubes and incubated in tissue culture flasks in the presence or absence of cisplatin (50 μM) for 1 h at 37°C and 5% CO₂. Immediately after incubation, PBLs were isolated using Polymorphprep as described above and stored at –20°C.

Experiments were also carried out to investigate the influence of incubation conditions on cisplatin adduct formation. In addition to the incubation of whole blood samples with cisplatin

Table 2 Characteristics of patients studied

Patient	Sex	Age (yrs)	BW ^a (kg)	SA (m ²)	Diagnosis	Chemotherapy	Cisplatin dose (mg/m ²)
1	F	3	11.9	0.56	Neuroblastoma	OPEC	80
2	M	3	15.1	0.65	Neuroblastoma	Rapid COJEC	80
3	F	1.4	8.1	0.42	Hepatoblastoma	PLADO	50
4	M	19	48.9	1.4	Osteogenic sarcoma	Cisplatin/adriamycin	100
5	F	7	19.3	0.78	Osteogenic sarcoma	Cisplatin/adriamycin	100
6	M	1.3	10.6	0.53	Neuroblastoma	OPEC/OJEC	80
7	F	15	63.8	1.7	Osteogenic sarcoma	Cisplatin/adriamycin	100
8	F	18	61	1.7	Osteogenic sarcoma	Cisplatin/adriamycin	100
9	M	11	60	1.7	Osteogenic sarcoma	Cisplatin/adriamycin	100
10	M	19	30.2	1.1	Osteogenic sarcoma	Cisplatin/adriamycin	100

^a BW, body weight; SA, surface area; OPEC, treatment with vincristine, cisplatin, etoposide, and cyclophosphamide; COJEC, treatment with vincristine, cisplatin, etoposide, and carboplatin; PLADO, treatment with cisplatin and doxorubicin; OPEC/OJEC, treatment with vincristine, cisplatin, etoposide, cyclophosphamide, and carboplatin.

as described above, additional blood samples (10 ml) were taken from seven healthy volunteers and PBLs isolated using Polymorphprep. These cells were resuspended in RPMI 1640 and incubated with cisplatin (50 μ M) in parallel with whole blood incubations. After this incubation period, cells were washed twice with PBS and the cell pellet stored at -20°C before analysis.

Pt-DNA Adduct Measurement. Cellular DNA was isolated from frozen PBL cell pellets as described previously (25) and the DNA concentration of each sample quantified by UV absorption (A_{260}). Pt-DNA adduct levels were determined by ELISA using a monoclonal antibody (ICR4) specific for cisplatin-induced adducts on DNA. The method used was based on that described previously (25) with modifications to the DNA plate-coating method and antibody dilution factor used to improve assay sensitivity. Adduct levels expressed in the results are determined by subtracting the control value, obtained from a pretreatment blood sample, from the actual adduct levels determined at 6, 24, and 48 h after the start of infusion for each individual patient. For *in vitro* studies, the control value was obtained from blood samples incubated in the absence of cisplatin for 1 h at 37°C and 5% CO_2 . Control values were calculated as 0.18 ± 0.10 nmol/g DNA (mean \pm SD). All of the samples were analyzed in duplicate in two separate experiments, and values are expressed as the average of these measurements. Duplicate Pt-DNA adduct values were within 20% of each other in all of the cases.

Pharmacokinetics. Pt pharmacokinetic analyses were carried out by flameless AAS using a Perkin-Elmer Analyst 600 graphite furnace spectrometer (Perkin-Elmer Ltd., Beaconsfield, United Kingdom). Total Pt concentrations were determined in plasma samples and free or unbound Pt levels determined in plasma ultrafiltrates as described previously (7, 12). All of the samples were analyzed in duplicate, and values are expressed as the average of these measurements. Duplicate values were within 15% of each other in all of the cases. Intra- and interassay coefficients of variation for a quality assurance sample had to be $<10\%$ for an assay to be valid. The limit of detection for the AAS was 0.10 $\mu\text{g/ml}$ for cisplatin standards.

Statistical Analysis. Linear regression analysis and the Pearson correlation coefficient were used to indicate correlations between pharmacokinetic parameters and Pt-DNA adduct

levels analyzed in this study. For statistical analysis of WBC toxicity, the logarithm of WBC count was used to determine correlations with adduct levels. The unpaired two-sided Student's *t* test was used to determine differences in WBC toxicity in subgroups of patients with high and low *in vivo* Pt-DNA adduct levels. The AUC and AUA were calculated between 0 and 48 h using the trapezoidal rule.

RESULTS

Cisplatin-DNA Adduct Measurements. PBLs isolated from blood samples taken before cisplatin administration and at 6, 24, and 48 h after the start of a 24-h infusion were analyzed for determination of *in vivo* Pt-DNA adduct levels. Fig. 1a shows that a similar pattern of adduct formation was observed in all of the 10 patients studied. DNA adduct levels were highest at 24 h, coinciding with the end of cisplatin infusion, and were higher 24 h after the end of infusion than in samples taken at 6 h after the start of infusion for all of the patients. DNA adduct levels ranged from 0.04 to 0.35 nmol/g DNA (median = 0.11) at 6 h, from 0.15 to 1.31 nmol/g DNA (median = 0.46) at 24 h, and from 0.12 to 0.88 (median = 0.35) at 48 h. No correlations were observed between the interpatient variation in Pt-DNA adduct levels and the age or gender of patients, as reported previously in an adult study (21).

To study Pt-DNA adduct formation *in vitro*, pretreatment whole blood samples from these 10 patients were incubated with cisplatin (50 μ M) for 1 h at 37°C before isolation of PBLs. A median Pt-DNA adduct level of 1.57 nmol/g DNA was observed with an ~ 4 -fold interpatient variation (range, 0.87–3.86) as shown in Fig. 1b. The influence of incubation conditions on cisplatin adduct formation in PBLs was investigated using blood samples obtained from seven healthy volunteers. Fig. 2 shows the relationship between adduct levels formed after the parallel incubation of cisplatin (50 μ M for 1 h) with either whole blood or isolated PBLs in RPMI 1640. No significant correlation was observed between these parameters ($r = 0.54$; $P = 0.2113$).

Pharmacokinetic Measurements. Plasma concentrations of unbound cisplatin measured in plasma ultrafiltrate samples showed an ~ 3 -fold interpatient variation in both C_{max} and AUC calculated using the trapezoidal rule. C_{max} values were observed at 24 h in all of the patients and ranged from 0.19 to

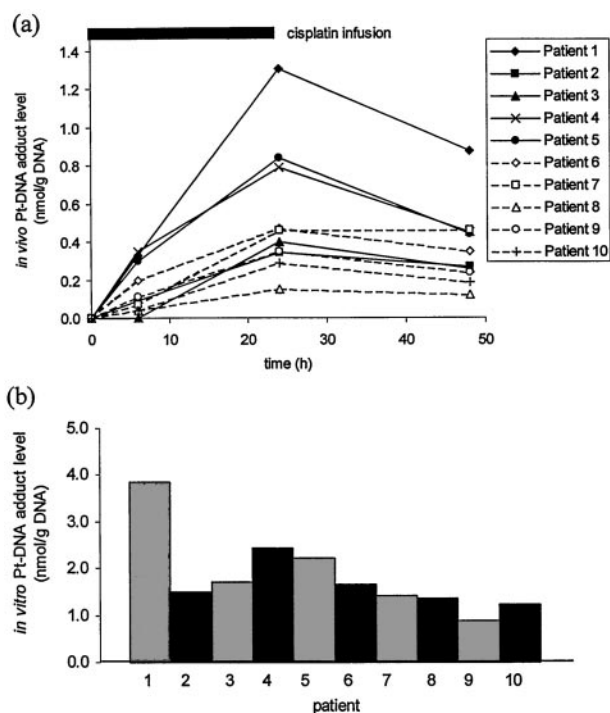


Fig. 1 Individual patient Pt-DNA adduct formation. a, Pt-DNA adduct-time curve obtained from 10 children after a 24-h infusion of cisplatin (mean ± SD). b, adduct levels determined in PBLs after a 1-h incubation of pretreatment blood with cisplatin (50 μM) in vitro. Adduct levels were measured in DNA extracted from PBLs isolated from fresh whole blood and all samples were analyzed in duplicate.

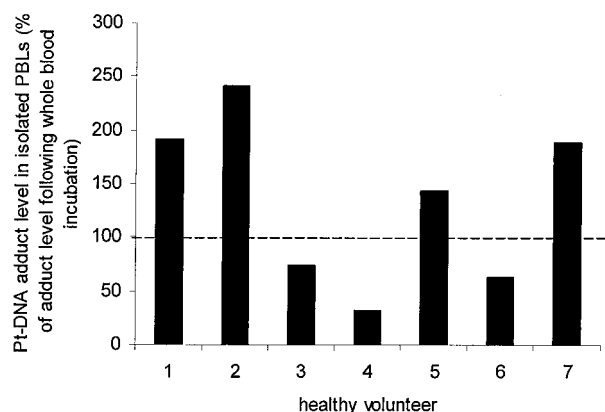


Fig. 2 Pt-DNA adduct levels after a 1-h incubation of isolated PBLs with cisplatin (50 μM) expressed as a percentage of the adduct levels formed after a 1-h incubation of whole blood with cisplatin (50 μM) from individual healthy volunteers.

0.52 μg/ml, with a median value of 0.35 μg/ml. Unbound cisplatin AUC_{0-48 h} values ranged from 378 to 1110 μg/ml/min, with a median value of 749 μg/ml/min.

Total cisplatin concentrations determined from plasma samples from 10 patients showed a similar 3-fold variation in C_{max} and AUC values. C_{max} values were again seen at 24 h

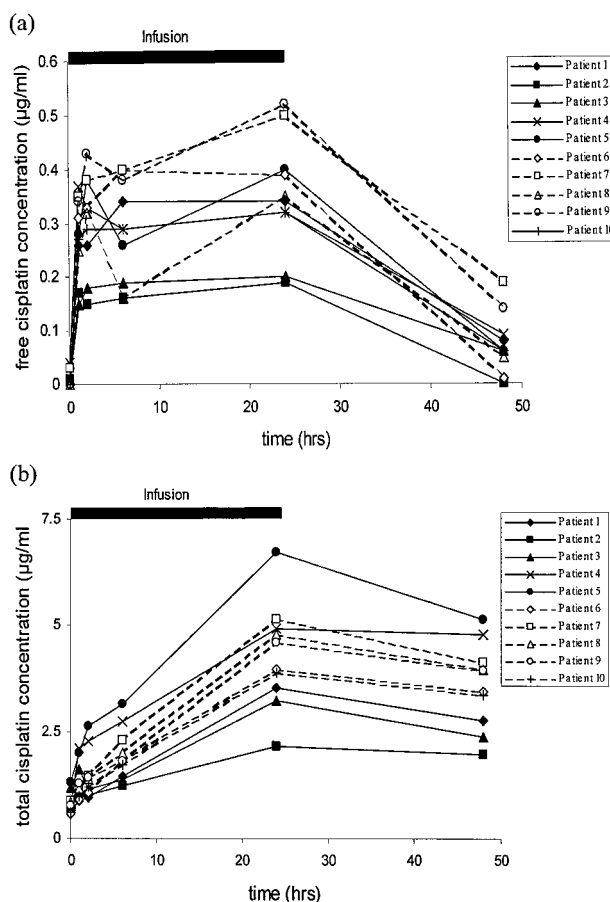


Fig. 3 Plasma concentrations of unbound (a) and total (b) Pt in 10 children after a 24-h infusion of cisplatin. Lines, data from individual patients.

corresponding with the end of cisplatin infusion and ranged from 2.17 to 6.74 μg/ml, with a median value of 4.29 μg/ml. Total cisplatin AUC_{0-48 h} values ranged from 5,201 to 14,833 μg/ml/min, with a median value of 9,526 μg/ml/min.

Fig. 3 shows individual unbound and total cisplatin time-curves for all of the patients studied. These data are comparable with similar studies concerning cisplatin pharmacokinetics in both adult and pediatric patients (15, 16).

Relationship between in Vitro and in Vivo Pt-DNA Adduct Levels.

To take into account the differences in cisplatin dose between individual patients, in vitro adduct levels were adjusted proportionately to the cisplatin dose administered before the relationship between in vitro and in vivo adduct levels was investigated. For example, the in vitro adduct level determined for patient 3, who received a cisplatin dose of 50 mg/m², was multiplied by 2 to be comparable with those patients who received a cisplatin dose of 100 mg/m² in vivo. This adjustment was based on previous studies, which have shown that there is a linear relationship between cisplatin concentration and formation of Pt-DNA adducts after incubation of whole blood with cisplatin in vitro (21). Similar experiments performed in our

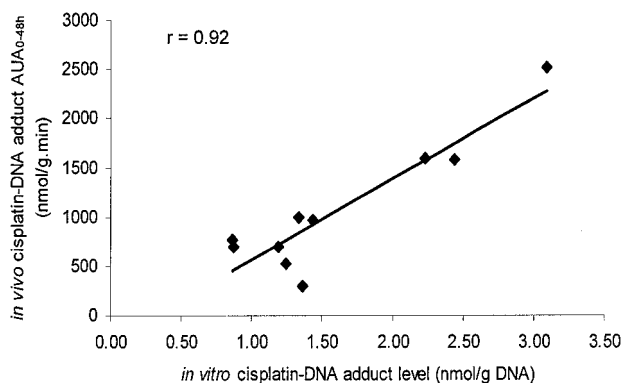


Fig. 4 Relationship between *in vivo* adduct formation expressed as AUA and *in vitro* adduct levels determined in PBLs after a 1-h incubation of pretreatment blood with cisplatin (50 μ M). \blacklozenge , individual patient data.

laboratory have shown a linear relationship up to a cisplatin concentration of 100 μ M.⁴

As shown in Fig. 4, a strong linear correlation ($r = 0.92$; $P = 0.0002$) was observed between Pt-DNA adduct levels determined after incubation of pretreatment whole blood with cisplatin *in vitro* and *in vivo* adduct formation expressed as AUA. Although the use of a correction for cisplatin dose seemed most appropriate, if the above correction was omitted, an equally strong correlation was seen between these two parameters ($r = 0.94$; $P < 0.0001$). The relationship between *in vitro* Pt-DNA adduct levels and *in vivo* adduct levels measured at individual time points was also investigated. The strongest correlation was seen with *in vivo* adduct levels at 24 h ($r = 0.92$; $P = 0.0002$), although similar relationships were also seen at 6 h ($r = 0.86$; $P = 0.003$) and 48 h ($r = 0.86$; $P = 0.002$).

Relationship between Cisplatin Pharmacokinetics and Pt-DNA Adduct Levels. Fig. 5 shows the relationship between cisplatin pharmacokinetics and *in vivo* adduct levels expressed as AUA after cisplatin treatment. No correlation was observed between *in vivo* adduct formation (AUA) and either unbound cisplatin AUC determined from ultrafiltrate samples ($r = 0.14$; $P = 0.69$) or total cisplatin AUC determined from plasma samples ($r = 0.18$; $P = 0.62$). In addition, no relationship was observed between adduct levels and cisplatin dose (data not shown).

Relationship between WBC Toxicity and Pt-DNA Adduct Levels. To assess potential differences in the degree of myelosuppression as a function of Pt-DNA adduct formation, total WBC counts determined after cisplatin treatment were related to cisplatin-DNA adduct levels (Table 3). Counts were taken as part of the routine clinical monitoring of these patients.

To investigate acute myelosuppressive effects, WBC counts on day 2 (*i.e.*, determined 24 h after the end of cisplatin infusion) were correlated with *in vivo* adduct levels expressed as AUA. WBC counts determined on day 2, expressed as a percentage of the pretreatment (day 0) count in individual patients,

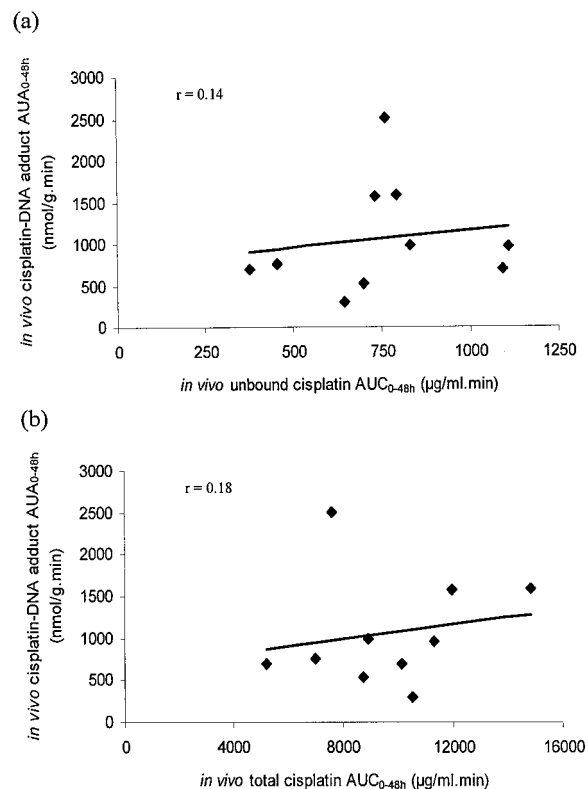


Fig. 5 Relationship between *in vivo* adduct formation expressed as AUA and exposure to unbound (a) and total (b) cisplatin expressed as AUC. \blacklozenge , individual patient data.

were shown to correlate inversely with AUA values ($r = -0.91$; $P = 0.0013$). Chronic myelosuppressive effects, which represent the leukopenia associated with cisplatin treatment, were assessed by determining WBC nadirs, which occurred in all of the patients between days 10 and 14 after treatment. The median value of AUA was used to separate the patients into two groups based on *in vivo* Pt-adduct exposure. A significant difference in WBC nadirs expressed as a percentage of pretreatment (day 0) counts (range, 2–90%) was observed between these subgroups of patients ($P = 0.010$) with greater WBC toxicity associated with higher levels of Pt-DNA adducts after cisplatin treatment ($AUA_{0-48\text{ h}} > 900$ nmol/g/min, mean WBC nadir = $10 \pm 7\%$ of day 0 count and $AUA_{0-48\text{ h}} < 900$ nmol/g/min, mean WBC nadir = $51 \pm 20\%$ of day 0 count). A significant correlation was also seen between Pt-DNA adduct levels after incubation of whole blood with cisplatin *in vitro* and WBC nadirs observed in all of the patients between days 10 and 14 after treatment ($r = -0.82$; $P = 0.0108$).

DISCUSSION

The development of sensitive assays to quantify Pt-DNA adducts has led to the publication of numerous studies investigating the role of adduct formation as a determinant of patient response to treatment with Pt agents. Results published by Reed *et al.* (18, 19) indicated positive correlations between response to cisplatin therapy and the extent of DNA adduct formation

⁴ Unpublished data.

Table 3 Acute and chronic WBC nadirs and Pt-DNA adduct levels in patients studied

Patient	AUA _{0-48h} ^a (nmol/g/min)	<i>In vitro</i> Pt-DNA adduct level (nmol/g DNA)	WBC count on day 2 (% of day/0 count)	WBC nadir between days 10–14 (% of day/0 count)
1	2512	3.86	39	17
2	694	1.49	90	90
3	763	1.72	90	46
4	1571	2.44	34	2
5	1591	2.23	ND	5
6	988	1.66	75	ND
7	961	1.43	184	16
8	304	1.36	280	37
9	693	0.87	240	40
10	531	1.24	373	41

^a AUA_{0-48h}, *in vivo* adduct formation expressed as AUA between 0 and 48 h after cisplatin administration; ND, not determined.

measured in PBLs in both ovarian and testicular cancer patients. Despite similar findings from studies involving patients with various different types of tumors, conflicting results have also been published. Studies by Motzer *et al.* (26) and Bonetti *et al.* (23) have indicated that no differences in adduct levels were evident between patients responsive and nonresponsive to cisplatin chemotherapy. There are several possible explanations for the contradictory data published including differences in the sensitivity of various tumor types to cisplatin therapy and potential differences resulting from the use of different assays to determine DNA adduct levels. Indeed, in a study published by Gupta-Burt *et al.* (27), a relationship was observed between adduct formation and clinical response using an ELISA technique to measure adduct levels but was not seen when samples were analyzed using an AAS method. However, the major concern when drawing conclusions from these studies is that in the majority of cases cisplatin was being used in combination with one or more additional anticancer drugs. In this scenario, the variation in Pt-DNA adduct formation determined in these patients may be no more important than differences in the molecular pharmacodynamics or pharmacokinetics of the concomitant chemotherapeutic agents.

Additional studies have been carried out in an attempt to identify the key factors controlling the extent of DNA adduct formation in a clinical setting. The results again appeared to be contradictory in a number of cases. Studies investigating potential relationships between *in vitro* and *in vivo* Pt-DNA adduct formation have shown positive correlations in two separate studies (21, 22), but no correlation was seen in a third, similar study (23). Despite the fact that these studies involved the use of three different analytical methods to determine adduct levels, which may entail the detection of different types of Pt-DNA adducts, the most likely reason for the conflicting data is an inconsistency in the experimental procedures used in investigating *in vitro* adduct formation. The study published by Bonetti *et al.* (23), which represents the only study to have shown a lack of correlation between *in vitro* and *in vivo* adduct formation, involved the incubation of freshly isolated PBLs with cisplatin as opposed to whole blood incubations in previous studies. This approach does not take into account the influence of factors in the blood, such as protein binding, that may affect adduct formation, and, additionally, the behavior of isolated PBLs when placed in an artificial medium may reflect the handling of

the cells during the isolation procedure. This issue has been addressed in the current study, and initial results indicate no relationship between Pt-DNA adduct levels in PBLs determined after incubation of whole blood with cisplatin and adduct levels from parallel incubations of isolated PBLs (Fig. 2).

More recently the influence of pharmacokinetics on the extent of Pt-DNA adduct formation have been investigated. In an adult patient population, a strong correlation between exposure to unbound cisplatin and adduct formation was suggested, and both were related to clinical response (16). This contrasts with results from a study in children that showed little or no correlation between pharmacokinetics and adduct levels (24). In addition to the distinct patient populations studied and the different assays used to quantify Pt adducts, these two studies also involved a significant difference in cisplatin infusion time (3 *versus* 24 h), which may have influenced their outcome.

The main drawback to the majority of studies published currently is that pharmacokinetic and pharmacodynamic factors were investigated separately, *i.e.*, the potential influences of these two key factors on Pt-DNA adduct formation were not compared in the same study. By studying both factors it is possible to remove variations in assay methods, patient populations, dosing schedules, or experimental differences in sample handling, all of which may have influenced, to a greater or lesser extent, the results obtained and the conclusions drawn from previous studies in this area.

Adduct levels determined in the current study were similar to those reported by Reed *et al.* (18, 19) using a different ELISA assay but lower than those reported previously by our group in a similar patient population (24). It has been reported by Ma *et al.* (28) that the use of frozen whole blood, as used in our previous study, could lead to erroneously high adduct levels attributable to persistence of active cisplatin during the isolation of PBLs. However, we presented results indicating that this was not a significant problem for our samples and methods (24). Because in the present study PBLs were isolated from fresh blood samples and lower adduct levels are reported, the results might be interpreted to support a difference between frozen and fresh blood samples. However, the lower adduct levels are more likely to be attributable to changes in the assay procedure between this and our previous study (see "Materials and Methods"). This possibility is indicated by the lower apparent adduct levels measured in the pretreatment samples compared with the

previous study (24). Because cisplatin was absent from such samples, this factor could not have influenced the results. This discrepancy in absolute adduct levels did not alter the conclusions drawn from these studies. Maximum levels of Pt-DNA adducts were observed at 24 h, coinciding with the end of cisplatin infusion, and declined gradually over the next 24 h. However, although the same pattern of adduct formation was seen in all of the patients studied, there was an 8-fold variation in overall adduct exposure expressed as AUA. Levels of adducts formed from *in vitro* incubations with cisplatin were ~3-fold higher than levels determined *in vivo*, which may be partly explained by the relatively high concentration of cisplatin (50 μM) used in the *in vitro* experiments. This concentration compares to a mean maximum plasma Pt concentration after cisplatin administration in these patients of 4.35 $\mu\text{g/ml}$ (14.5 μM). Cisplatin pharmacokinetics determined in the present study were similar to those reported previously in a similar pediatric patient population (15) and showed an ~10-fold greater exposure to total cisplatin measured in the plasma than to unbound cisplatin measured in plasma ultrafiltrate samples. Both total and unbound cisplatin plasma concentrations exhibited an ~3-fold interpatient variability.

The data obtained from this study were analyzed to investigate the role of cisplatin pharmacokinetics in determining Pt-DNA adduct formation in parallel with investigating the potential relationship between *in vitro* and *in vivo* adduct formation. A strong correlation ($r = 0.92$) was observed between DNA adduct levels determined after incubation of pretreatment whole blood with cisplatin *in vitro* and *in vivo* adduct formation expressed as AUA. In comparison, no significant correlation was observed between *in vivo* adduct formation (AUA) and either unbound cisplatin AUC ($r = 0.14$) or total cisplatin AUC ($r = 0.18$). Whereas this study involves a relatively small number of patients, the pharmacokinetic data build on that published previously from our group in a very similar pediatric patient population using an identical cisplatin-dosing schedule (24). This now gives us a total of 27 patients studied with no indication of any correlation between cisplatin pharmacokinetics and Pt-DNA adduct formation. The data indicating a strong correlation between *in vivo* adduct levels and levels determined after *in vitro* incubation of pretreatment blood samples supports similar results published from adult studies (21, 22).

To assess potential differences in the degree of myelosuppression as a function of Pt-DNA adduct formation, WBC counts were measured after cisplatin treatment and related to *in vivo* adduct formation. A close correlation was seen between the percentage decrease in WBC counts determined 48 h after cisplatin treatment and AUA values ($r = -0.91$). Whereas this supports the role played by adduct formation in determining the cytotoxic effects of cisplatin, it does not necessarily have a significant clinical relevance, because the leukopenia associated with cisplatin therapy is commonly seen up to 2 weeks after treatment. However, WBC nadirs observed in patients between days 10 and 14 after treatment, corresponding to the myelosuppressive effects of cisplatin, were also related to Pt-DNA adduct levels. Using the median value of AUA as a cutoff level to define patients with "high" and "low" *in vivo* Pt-adduct levels, a significant difference was observed in WBC nadirs expressed as a percentage of pretreatment counts between these patient

groups ($P = 0.010$), with higher adduct levels corresponding to greater WBC toxicity. A correlation was also observed between adduct formation in pretreatment blood samples incubated with cisplatin *in vitro* and the extent of myelosuppression seen in individual patients after cisplatin treatment.

In summary, this study demonstrates that the formation of Pt-DNA adducts in PBLs of children receiving cisplatin treatment is determined by host-specific factors and is not simply a function of cisplatin pharmacokinetics. This may involve variations in drug uptake into PBLs, intracellular inactivation of cisplatin, or the influence of factors in the blood, which may affect drug availability or uptake. The latter would explain the lack of correlation between adduct formation in PBLs after the incubation of cisplatin with whole blood *versus* isolated cells. These data suggest that the measurement of adduct levels in PBLs after incubation of pretreatment whole blood samples with cisplatin may be used to predict *in vivo* adduct formation in individual patients. Taken in conjunction with studies indicating correlations between adduct levels and patient response, it is feasible that *in vitro* adduct levels could be used as a predictive marker of response to chemotherapy. This may allow the identification of patients who are less likely to obtain a significant clinical benefit from cisplatin treatment because of low levels of adduct formation. Similarly, patients with an increased likelihood of experiencing side-effects from cisplatin treatment, such as the myelosuppressive effects investigated in the current study, may be identified. To substantiate this relationship, studies investigating the influence of pharmacokinetic and cellular effects in parallel would have to be expanded to a larger patient cohort. However, in summarizing data from this and related work, it is clear that the key component lacking in all of these studies is Pt-DNA adduct formation in tumor material. If a link could be demonstrated between adduct formation in PBLs and tumor material in patients receiving cisplatin treatment, this would add significant weight to the findings of many studies published in this area. Studies addressing this key issue are currently being undertaken in our laboratory.

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