Cancer Chemother Pharmacol (1996) 39: 25–33

ORIGINAL ARTICLE

Anders Johnsson · Peter Höglund · Anders Grubb
Eva Cavallin-Ståhl

Cisplatin pharmacokinetics and pharmacodynamics in patients with squamous-cell carcinoma of the head/neck or esophagus

Received: 5 November 1995/Accepted: 15 May 1996

Abstract The pharmacokinetics of total platinum (Pt) in plasma and cisplatin (CDDP)-DNA adducts in different cell types were described in ten patients with squamous-cell carcinoma of the head/neck or esophagus after their first cycle of chemotherapy containing a CDDP dose of 100 mg/m^2. Nephrotoxicity was studied in terms of urinary excretion of marker proteins (protein HC, IgG, and albumin). Pharmacodynamic relationships between pharmacokinetic parameters and toxicity were investigated. A population-based model with limited sampling was found feasible for producing pharmacokinetic information, in accordance with literature data. The kinetics of two normal cell types with different turnover (lymphocytes and buccal cells) appeared to have different kinetic profiles of CDDP-DNA adducts. Analysis of urinary excretion of marker proteins (protein HC, albumin, and IgG) showed that the nephrotoxicity was displayed first as tubular damage and later as impaired glomerular barrier function. There were indications that tubular nephrotoxicity may be predicted by pharmacokinetic parameters of plasma Pt. We found older patients to have a lower Pt clearance and more extensive early tubular damage. There was no correlation between CDDP-DNA adducts in normal cells and nephrotoxicity. Larger studies are warranted to define the pharmacokinetic window of CDDP. Limited sampling for analysis of CDDP pharmacokinetics may then be a possible avenue for individualizing the dose and, thus, improving the clinical use of the drug.

Key words CDDP · Pharmacokinetics · pharmacodynamics · CDDP-DNA adducts · Nephrotoxicity

A. Johnsson (✉) · E. Cavallin Ståhl
Department of Oncology, University Hospital, S-22185 Lund, Sweden
Fax: + 46 + 46-147327

P. Höglund
Department of Clinical Pharmacology, University Hospital, Lund, Sweden

A. Grubb
Department of Clinical Chemistry, University Hospital, Lund, Sweden

Introduction

Cisplatin [cis-diamminedichloroplatinum(II), CDDP] is a potent anticancer agent for treatment of a variety of cancers, e.g., testicular, ovarian, bladder, head/neck (H/N), and esophageal cancer. CDDP has substantial toxicity. One of its most frequent and serious side effects is nephrotoxicity. Dosing of most chemotherapeutic drugs, including CDDP, is based on the body surface area of the patient. This is a crude way to calculate the dose, since individual differences in the distribution and elimination of the drug are not taken into account. The relationships between the drug's pharmacokinetics and its antitumor effects and side effects are incompletely known. The pharmacokinetics of CDDP is usually monitored as platinum (Pt) concentrations in plasma occurring as either total Pt or ultrafiltrable, free Pt. CDDP is thought to act by binding to DNA and thus forming CDDP-DNA adducts. Pharmacodynamic correlations have been found between total plasma Pt and nephrotoxicity [4, 8] and tumor response [8]. Correlations have also been demonstrated between CDDP-DNA adducts in white blood cells [11, 26] or buccal cells [3] and tumor response.

Monitoring of renal toxicity induced by CDDP treatment is usually performed by analysis of serum creatinine levels or creatinine clearance. However, these two parameters assess only the glomerular filtration rate and cannot demonstrate tubular damage or impaired glomerular barrier function. A more careful monitoring of kidney toxicity, therefore, requires

measurement of the urinary excretion of specific marker proteins in addition to determinations of parameters for the glomerular filtration rate [14, 15].

The main objective of the present study was to describe CDDP pharmacokinetics in terms of total plasma Pt measurements and CDDP-DNA adduct analysis in different cell types in a group of cancer patients after their first cycle of CDDP. A further objective was to characterize the early CDDP-induced kidney damage by analysis of urinary excretion of proteins and to investigate correlations between the pharmacokinetics and the renal toxicity of the drug.

Patients and methods

Patients and treatment

Ten consecutive patients who had been referred to the Department of Oncology at the University Hospital of Lund with pathologically confirmed squamous-cell carcinoma of the H/N or esophagus were included in the study (Table 1). All patients had locoregional disease without signs of distant metastates. Nine of the patients had newly diagnosed cancer and one of the patients with laryngeal cancer had experienced a recurrence after previous radiotherapy. No patient had received prior chemotherapy. There were seven men and three women aged a median of 59 years (range 44–74 years). All had a performance status of < 1 on the WHO scale, normal serum creatinine values (ranging from 47 to 83 μmol/l), and normal blood counts.

All patients were assigned to receive three courses of induction chemotherapy at 3-week intervals, followed by external radiotherapy (40–66 Gy, depending on the site) and, in three cases, surgical resection of the primary tumor or regional lymph nodes. Each course of chemotherapy consisted of 100 mg/m^2 of CDDP given on day 1 and 5-fluorouracil (5-FU) given on days 1–5 (750 or 1000 mg/m^2 as a continuous 72-h infusion). The CDDP was dissolved in 1000 ml of normal saline and was given as a continuous infusion over a planned infusion time of approximately 4 h. Forced diuresis was maintained by pre- and posthydration with infusions of normal saline and mannitol. All patients were treated in the same unit at the Department of Oncology, University Hospital, Lund, Sweden.

Sampling

For the pharmacokinetic analyses, blood samples and buccal cells were collected before the start of CDDP treatment (-4 h), at the end of the infusion (0 h), and at 2 and 20 h after the end of the infusion during the first treatment course. Approximately 10 ml of blood was drawn into heparinized tubes at every time point. A small portion (2 ml) was centrifuged immediately for 5 min at 1500 rpm to obtain plasma, which was frozen at $-70\,^{\circ}$C for later Pt analysis. The remainder was subjected to gradient centrifugation on Lymphoprep (Nycomed AS, Oslo, Norway). The isolated mononuclear cell fraction was frozen in Tissue Teck embedding medium (Histolab Products AB, Gothenburg, Sweden) and was stored at $-70\,^{\circ}$C until the preparation of 10-μm-thick cryosections on poly-L-lysine-coated slides for analysis of CDDP-DNA adducts. Buccal cells were collected from the mucosa of the inner side of the cheek by repeated wiping with a cytology brush. The cells were transferred into Hanks' balanced salt solution (Sigma, St. Louis, Mo., USA) and washed twice in NaCl. Cytospin slides were prepared (5 min at 500 rpm) on glass sides coated with poly-L-lysine. The slides were stored at $-70\,^{\circ}$C for later analysis of CDDP-DNA adducts. In two patients with ulcerated oral cancers, tumor cells were also sampled with a cytology brush at the same time points and prepared in the same manner as the buccal cells.

Urine samples were collected at -4 h, at 20 h, and at 3 weeks after CDDP treatment and were used for determination of selected proteins and creatinine. All analyses were performed within 24 h of collection and without prior freezing of the samples.

Table 1 Patients' characteristics and tumor-response estimation (*CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable)

Patient number	Sex	Age (years)	Tumor site	Tumor response
1	M	59	Larynx	CR
2	M	74	Hypopharynx	CR
3	M	72	Esophagus	NE
4	F	55	Oropharynx	CR
5	M	44	Oral cavity	PR
6	M	48	Esophagus	SD
7	F	73	Esophagus	NE
8	M	59	Oropharynx	PR
9	F	62	Larynx[a]	PD
10	M	52	Oral cavity	PR

[a] Recurrent disease

Pt analysis

Pt concentrations in plasma were determined by flameless atomic absorption spectrophotometry (FAAS). Sample preparation and heating temperatures have previously been described elsewhere [18]. Measurements were made with a Varian Spectra AA-40Z spectrophotometer with Zeeman background correction.

CDDP-DNA adduct analysis

An immunohistochemical staining technique was used for visualization of CDDP-DNA adducts in lymphocytes, buccal cells, and tumor cells. Slightly different staining protocols were used for the cryosectioned lymphocytes [17, 31] and the cytospinned buccal cells [32]. The NKI-A59 antiserum (a gift from Drs. L. den Engelse and F.A. Blommaert, the Netherlands Cancer Institute, Amsterdam) elicited against CDDP-DNA interaction products was used [31]. A brown nuclear staining reaction was developed by use of the peroxidase-antiperoxidase complex and diaminobenzidine. Methyl green was used as a nuclear counterstain.

The staining reaction was quantitated with the computerized image analyzer CAS 200 (Cell Analysis System, Elmhurst, Ill, USA) using a two-color mask image technique with the quantitative nuclear antigen (QNA) software package. On each slide, 50–100 morphologically intact cells were analyzed. Results of the measurements were given as the percentage of positive nuclear area (PNA). This procedure has been described and evaluated in a previous report [17].

Urinary protein analysis

Renal dysfunction can be classified into different categories by the quantitation of urinary proteins [14–16]. Increased excretion of albumin and IgG indicate glomerular damage with impaired barrier

function, whereas increased urinary excretion of free protein HC (alias alpha$_1$-microglobulin) is a sensitive marker for proximal tubular dysfunction. In the present study, concentrations of albumin, IgG, and protein HC were determined by automated immunoturbidimetric techniques as previously described [30]. Urinary creatinine levels were determined by the Kodak Ektachem 700 XR-C system, which uses the enzyme creatine amidinohydrolase. Protein concentrations were expressed as milligrams of protein per millimole of creatinine to adjust for individual and temporal differences in diuresis. Reference values were obtained from a healthy population of 97 adults.

Pharmacokinetic calculations

The elimination of total Pt after an infusion of CDDP has been shown to occur with two or three exponential terms [34], depending on the duration of infusion and the statistical model used [12, 21, 34]. In the present study a two-compartment model was chosen. Due to the sparse concentration data, we used a population approach based on the ten patients. NONMEM computer software [1, 2] was utilized for calculation of clearance (CL), of volumes of distribution in the central (Vd) and peripheral compartments, and of clearance between the central and peripheral compartments. Mean parameters of the population were calculated and interindividual variation was estimated.

Individual parameters were calculated as empirical Bayes estimates on the basis of the individual concentrations and the population parameters. These estimates were used to create plots of the individual profiles (Fig. 1). The parameters used for the pharmacokinetic and pharmacodynamic correlations were the individual values for the volume of distribution in the central compartment (Vd), the terminal half-life ($t_{1/2}$), clearance (CL), and the area under the concentration-time curve (AUC). The AUC value was calculated as the ratio of dose and CL.

Statistical methods

Correlations between parametric variables were calculated as Pearson's correlation coefficients.

Results

Plasma platinum kinetics

The actual duration of CDDP infusion ranged between 3.3 and 6 h. The mean (SD) population parameters obtained from NONMEM were CL 0.27 (0.19) $l\,h^{-1}\,m^{-2}$ and Vd 13.1 (4.5) l/m^2. The mean volume at steady state was 23.4 l/m^2 and the intercompartmental clearance was 7.6 $l^{-1}\,h^{-1}\,m^{-3}$. The results were used for Bayesian estimation of individual profiles (Fig. 1) and parameters (Table 2). The mean (SD) observed peak concentration (C_{max}) was 2.9 (0.6) µg/ml and the calculated parameters were Vd 24.1 (9.7) l, CL 0.53 (0.35) l/h, $t_{1/2}$ 93.6 (66.9) h, and AUC 347 (278) $mg\,h\,l^{-1}$.

CDDP-DNA adduct kinetics

The kinetic profiles, expressed as mean PNA values (Fig. 2), were different in the two normal cell types.

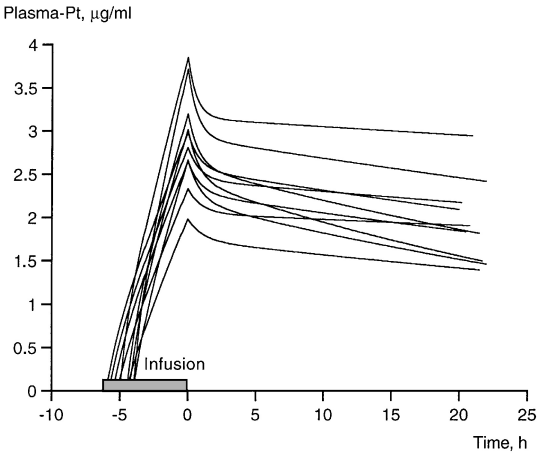

Fig. 1 Plasma-Pt profiles estimated in ten patients after the first treatment with a CDDP dose of 100 mg/m^2

Formation of CDDP-DNA adducts occurred early in buccal cells, with the peak level being measured at the end of the CDDP infusion, whereas the adduct level in lymphocytes increased gradually during the study period of 20 h. Tumor cell adducts in the two patients studied seemed to occur rapidly, with peaks being observed early after the end of the infusion, followed by a gradual decline (Fig. 3).

In all cell types there were varying degrees of nonspecific nuclear staining in the pretreatment samples (−4 h), with large interindividual variations. To correct for this, the PNA value recorded at −4 h was subtracted from the PNA values obtained at the other time points (0, 2, and 20 h) in each patient. The following comparisons were based on corrected PNA values. There was no significant correlation between C_{max} or AUC values and the levels of CDDP-DNA adducts found in the two cell types at any time point.

Factors potentially affecting the pharmacokinetics

Older patients had a longer $t_{1/2}$, a lower CL, and, consequently, a larger AUC (Table 3). Pretreatment serum creatinine and serum albumin levels did not affect the pharmacokinetic parameters in terms of either Pt concentrations or CDDP-DNA adduct levels in lymphocytes or buccal cells (Table 3). The actual infusion time had no impact on the pharmacokinetics.

Nephrotoxicity

The urinary excretion of protein HC increased rapidly after cessation of the CDDP infusion (Fig. 4), with eight of 10 patients having elevated levels (>1 mg/mmol) after 20 h and six of those having a pronounced

Table 2 Pharmacokinetic parameters of total plasma Pt and peak levels of CDDP-DNA adducts as determined in ten patients after the first treatment with a CDDP dose of 100 mg/m^2 (C_{max} Peak Pt concentration, *Vd* volume of distribution, *CL* Pt clearance, $t_{1/2}$ terminal half-life, *AUC* area under the concentration-time curve, *Ly* lymphocytes, *Bu* buccal cells, *PNA* percentage of positive nuclear area – corrected values)

Patient number	Infusion time (h)	Pt plasma pharmacokinetics				CDDP-DNA adducts		
		C_{max} (μg/ml)	Vd (I)	CL (I/h)	$t_{1/2}$ (h)	AUC (mg h l^{-1})	Ly peak (PNA)	Bu peak (PNA)
1	4.0	3.80	17.7	0.38	80.2	348	21	14
2	5.2	2.25	32.3	0.19	202.0	604	54	23
3	6.0	3.05	19.7	0.43	68.5	264	2	9
4	4.5	3.25	15.1	0.57	41.6	165	7	11
5	4.0	2.70	33.9	1.18	37.7	125	13	40
6	4.5	1.90	44.4	0.71	68.1	176	47	21
7	4.5	3.85	13.8	0.12	218.3	1004	0	28
8	5.8	2.70	20.3	0.23	123.4	443	2	28
9	5.5	2.50	22.1	0.45	64.9	222	0	43
10	3.3	2.95	21.2	1.04	30.8	116	29	39
Mean (SD)		2.90 (0.63)	24.1 (9.7)	0.53 (0.35)	93.6 (66.9)	347 (278)		

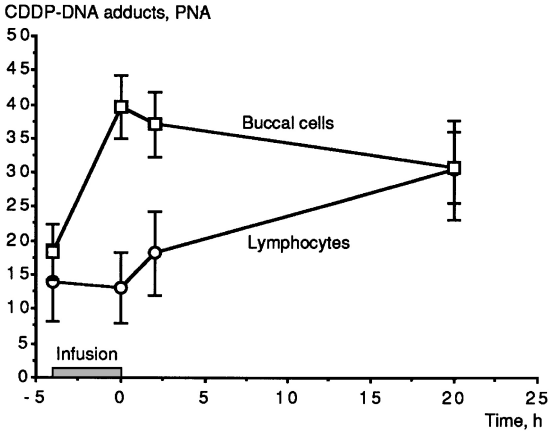

Fig. 2 CDDP-DNA profiles in lymphocytes and buccal cells as determined in ten patients after the first treatment with a CDDP dose of 100 mg/m^2. Data represent mean values ± SE (*PNA* percentage of positive nuclear area)

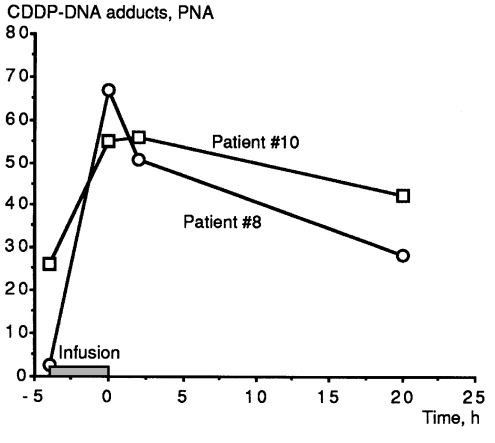

Fig. 3 CDDP-DNA profiles in tumor cells as determined in two patients after the first treatment with a CDDP dose of 100 mg/m^2 (*PNA* percentage of positive nuclear area)

elevation (> 3 mg/mmol). By 3 weeks the mean urinary protein HC concentration had declined, but six of nine patients continued to display elevated levels. The temporal changes in the urinary excretion of albumin and IgG differed markedly from those of protein HC, with a gradual increase being seen in the mean levels of albumin and IgG as well as in the number of patients with abnormal values at between 20 h and 3 weeks (Figs. 5, 6). There was a significantly positive correlation between age and protein HC concentration at 20 h ($r = 0.74$, $P = 0.01$), but not at 3 weeks. No significant correlation was found between age and the levels of other investigated urinary proteins.

The pharmacodynamic analysis showed a significant correlation between the C_{max} value and the concentration of protein HC at 3 weeks. A low CL correlated with a high protein HC level at 20 h (Table 4). All six patients with markedly elevated levels of protein HC (> 3 mg/mmol) at 20 h had a CL below 0.5 l/h and an AUC value above 200 mg h l^{-1}. Two of those patients had a persistent elevation of protein HC above 3 mg/mmol at 3 weeks, and they also developed kidney dysfunction with serious clinical implications. One patient had uremia with septicemia after three courses of chemotherapy and the other patient had a marked but transient increase in serum creatinine levels after the first CDDP treatment, leading to withdrawal from further chemotherapy. No significant correlation was found between the other pharmacokinetic parameters and the levels of urinary proteins (Table 4).

Pretreatment serum creatinine values did not correlate with the urinary protein levels determined at any time point after CDDP treatment, but it should be noted that all patients had pretreatment serum creatinine values in the normal range. A slightly elevated concentration of urinary proteins was seen in very few patients before treatment (Figs. 4–6), and there was no

Table 3 Correlations between "predisposing factors" and pharmacokinetics (C_{max} peak Pt concentration, *Vd* volume of distribution, *CL* Pt clearance, $t_{1/2}$ terminal half-life, *AUC* area under the concentration-time curve, *Ly* lymphocytes, *Bu* buccal cells, *PNA* percentage of positive nuclear area – corrected values)

	Pt plasma pharmacokinetics					CDDP-DNA adducts	
	C_{max}	Vd	CL	$t_{1/2}$	AUC	Ly peak PNA	Bu peak PNA
Age	+ 0.28	− 0.43	− 0.83*	+ 0.74*	+ 0.73*	− 0.14	− 0.32
S-albumin	+ 0.13	− 0.07	+ 0.18	− 0.19	− 0.17	− 0.04	+ 0.07
S-creatinine	− 0.06	+ 0.38	+ 0.04	+ 0.35	+ 0.24	+ 0.43	− 0.04
Infusion time	− 0.27	− 0.07	− 0.62	+ 0.31	+ 0.21	− 0.28	+ 0.07

*$P < 0.05$ (statistically significant correlation)

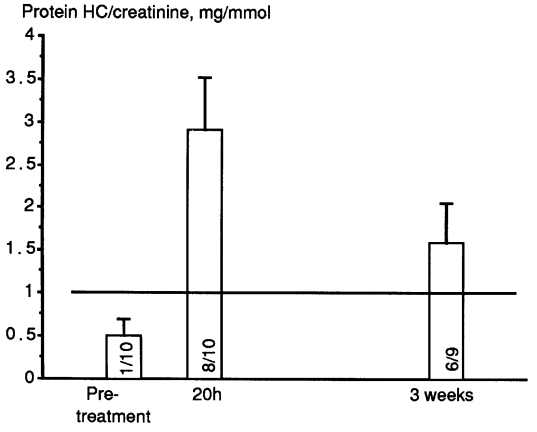

Fig. 4 Urinary protein HC/creatinine levels measured in ten patients before treatment as well as at 1 day and at 3 weeks after the first treatment with a CDDP dose of 100 mg/m^2. The *bars* indicate mean values + SE. The *horizontal line* shows the upper limit of the normal value. *Numbers in the bars* indicate the number of patients with a value above the normal limit/the number of patients at risk

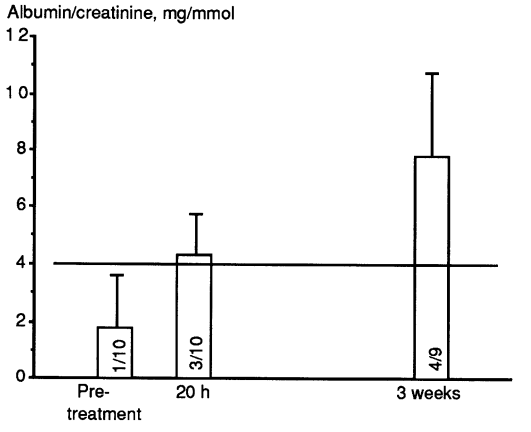

Fig. 5 Urinary albumin/creatinine levels measured in ten patients before treatment as well as at 1 day and at 3 weeks after the first treatment with a CDDP dose of 100 mg/m^2. The *bars* indicate mean values + SE. The *horizontal line* shows the upper limit of the normal value. *Numbers in the bars* indicate the number of patients with a value above the normal limit/the number of patients at risk

obvious relationship between the pretreatment and the posttreatment levels of urinary proteins.

Clinical observations

Five of the ten patients received three courses of chemotherapy as planned. Three patients had two cycles and two proteins had only one cycle of CDDP + 5-FU. The reasons for premature cessation of chemotherapy were cardiac toxicity, including asymptomatic ST changes on the electrocardiogram (one patient) and arrhythima of WHO grade 2 (one patient); nephrotoxicity of WHO grade 2 (one patient); progressive disease (one patient); and general fatigue (one patient).

Evaluation of the tumor response was not included as a primary objective of this study. All patients continued with radiotherapy either shortly after the completion of chemotherapy or concomitantly with third cycle of chemotherapy, which made an evaluation of the chemotherapeutic effect very difficult. With these

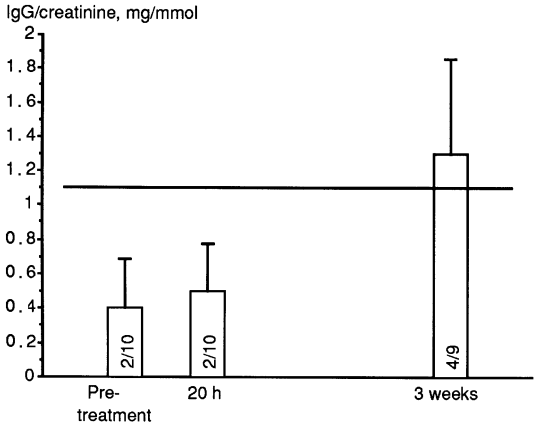

Fig. 6 Urinary IgG/creatinine levels measured in ten patients before treatment as well as at 1 day after and at 3 weeks after the first treatment with a CDDP dose of 100 mg/m^2. The *bars* indicate mean values + SE. The *horizontal line* shows the upper limit of the normal value. *Numbers in the bars* indicate the number of patients with a value above the normal limit/the number of patients at risk

Table 4 Correlations between pharmacokinetic and toxicity parameters (C_{max} peak Pt concentration, Vd volume of distribution, CL Pt clearance, $t_{1/2}$ elimination half-life, AUC area under the concentration-time curve, Ly lymphocytes, Bu buccal cells, W weeks)

	Pt plasma pharmacokinetics					CDDP-DNA adducts	
	C_{max}	Vd	CL	$t_{1/2}$	AUC	Ly peak	Bu peak
U-Protein HC 20 h[a]	− 0.26	− 0.49	− 0.77*	+ 0.48	+ 0.50	− 0.53	− 0.13
U-Protein HC 3 w[a]	+ 0.71	− 0.74*	− 0.32	− 0.14	+ 0.04	− 0.48	− 0.51
U-Albumin 20 h[a]	− 0.04	− 0.38	− 0.37	+ 0.04	+ 0.04	− 0.47	+ 0.36
U-Albumin 3 w[a]	+ 0.54	− 0.62	− 0.26	− 0.17	+ 0.06	− 0.35	− 0.43
U-IgG 20 h[a]	+ 0.01	− 0.18	− 0.10	− 0.25	+ 0.22	− 0.37	+ 0.21
U-IgG 3 w[a]	+ 0.19	− 0.53	− 0.24	− 0.17	+ 0.10	− 0.55	− 0.33

* $P < 0.05$ (statistically significant correlation)
[a] Analyzed in urine

insufficiencies in mind, we undertook an estimation of the tumor response. Eight patients were evaluable for response after a minimum of two cycles of chemotherapy. There were six responders (three complete and three partial responses) and two nonresponders (one with stable disease and one with progressive disease; Table 1).

Discussion

We studied several aspects of pharmacokinetics and pharmacodynamics in a group of cancer patients treated with a CDDP-containing chemotherapy regimen. This is, to our knowledge, the first investigation to describe correlations between Pt pharmacokinetics and nephrotoxicity in terms of the urinary excretion of marker proteins. The present study was small with regard to the number of patients. On the other hand, the material was relatively uniform in that all patients were chemotherapy-naive and were treated with the same chemotherapy regimen under standardized treatment conditions.

CDDP binds rapidly to plasma proteins [33]. After 24 h, more than 95% of CDDP is protein-bound [6, 22, 23, 33]. The plasma pharmacokinetics of CDDP can be studied in terms of total Pt or as non-protein-bound, ultrafiltrable Pt. The latter gives a better measure of the active portion of Pt in plasma, and it can therefore be argued that analysis of ultrafiltrable Pt is more relevant than that of total Pt. Clinical studies have shown correlations between ultrafiltrable Pt and nephrotoxicity [25] as well as with the tumoricidal effect [27], but total plasma Pt has also been shown to correlate with nephrotoxicity [4, 8, 20] and efficacy [8]. A recent study [13] has shown a very close linear relation between free and total plasma Pt at various time points after CDDP treatment. This observation suggests that the free Pt pool constitutes a constant portion of total plasma Pt and that the protein binding of CDDP may not be irreversible as is usually reported. This equilibrium between free and total Pt also indicates that total plasma Pt may be used as a pharmacokinetic marker for the amount of active CDDP. Furthermore, analysis of total Pt is technically easier to perform, which may be advantageous for the reliability of the method [8]. Therefore, we chose to study the pharmacokinetics in terms of total plasma Pt.

Several studies have previously described the pharmacokinetics of total Pt [12, 21, 28, 33]. There is a considerable interstudy variation in the kinetic parameters presented, possibly due to differences in the duration of infusion, sampling time points, Pt assay, and pharmacokinetic model used. The results of the present study fall in the range of these previous reports, in spite of the small number of patients and sampling time points involved. This indicates that a population-based model with limited samples may provide useful pharmacokinetic information.

The kinetics of CDDP-DNA adducts were different in the two different normal cell types studied. The formation of adducts occurred rapidly in buccal cells, followed by a slow decline during the first 20 h, whereas the adduct formation in lymphocytes was a slower process, with a gradual increase occurring during the first 20 h. This discrepancy probably reflects a higher turnover of buccal cells as opposed to lymphocytes. The oral mucosa is a rapidly regenerating tissue, whereas circulating lymphocytes have a very long average life span. There may also be differences in the capacity to repair DNA damage in the two cell types. In one previous study, CDDP-DNA adduct kinetics have been analyzed in lymphocytes [29], with similar adduct levels being reported directly after and at 20 h after CDDP treatment. These results were partly in agreement with those of our study in that there was no decrease in lymphocyte adducts during the 1st day after CDDP injection. Most previous studies [5, 11, 26] on adducts in blood cells have been made on DNA from the whole pool of the nucleated blood cells, mainly consisting of granulocytes, which have a very short half-life. The adducts in leukicytes have been shown to decrease during the 1st day after CDDP treatment

[11], possibly due to the high turnover of that cell population. Buccal cell adducts have been shown to appear soon after treatment [32] and to be elevated at 24 h posttreatment [3], but their kinetics during the 1st day has not previously been characterized. The adduct kinetics in tumor cells, which was studied in two patients in the present study, seemed quite similar to the buccal cell profile. This similarity might be explained by the observation that tumor cells, like buccal cells, have a high proliferation rate. The study of CDDP-DNA adducts in tumors should be of great clinical value, since it is a way of viewing the drug in its "ultimate" target position. The immunohistochemical technique should be a suitable approach for analysis of tumor cell adducts because it allows visual discrimination of intact tumor cells from irrelevant necrotic and inflammatory cell components.

The differences observed in the kinetics of the CDDP-DNA adducts in lymphocytes and buccal cells, along with the lack of correlation found between adduct levels in the two cell types, indicate that the choice of cell type and the sampling time points are compound factors that should be carefully considered in the design of study protocols that include adduct analyses in normal cells.

One would have expected a positive correlation between the pharmacokinetics in terms of total Pt and CDDP-DNA adducts in normal cells, which was not found in the present study. There may be methodological reasons for this, such as technical problems in obtaining representative buccal cell samples. Several studies have shown substantial variation in the levels of adducts in leukocytes between individuals [10, 11, 26]. It may be that large interindividual differences in the capability to form and repair adducts may blur a possible relationship to the pharmacokinetic parameters.

The age of the patient affected the pharmacokinetics. Older patients had a lower CL, which increased the half-life and the AUC. This was probably due to the general impairment of kidney function that occurs with increasing age. Our findings contrast with the results of a previous study [34] in which no such correlation was found. This could be explained by the observation that the patients in our study were older than those in the study by Vermorken et al. [34], the difference in median age being 9 years. We also found older patients to have a greater risk of developing early tubular kidney damage.

The present study showed no correlation between serum albumin levels and the Pt pharmacokinetics, in agreement with one previous report [9] but in contrast to others that found correlations between serum albumin values and the total Pt half-life [28, 34]. The reason for this discrepancy may be that most serum albumin measurements obtained in the present study were normal, with only two patients having a value below the normal range. The pretreatment level of serum creatinine did not affect the pharmacokinetics, in accordance with the results of several previous studies [9, 19, 34].

The variations in infusion time, ranging from 3.3 to 6 h in our study, had no impact on the pharmacokinetic parameters. This finding was in accordance with that reported by Stewart et al. [28], who found similar pharmacokinetics within a much wider range of infusion duration, i.e., from < 1.5 to > 20 h. Thus, the limited deviations from the planned infusion schedule that sometimes occur due to technical or practical problems do not seem to influence the pharmacokinetics and probably do not affect the outcome of the treatment either.

Nephrotoxicity is a major side effect of CDDP treatment. Analysis of the excretion of selected urinary proteins allows the sensitive demonstration of nephrotoxicity and its characterization as glomerular and/or tubular [14–16]. The tubular damage as reflected by protein HC excretion was observed as early as 20 h after the first CDDP treatment, whereas the glomerular damage appeared later, in accordance with previous reports [7, 24]. This indicates different underlying mechanisms. We [18] and other investigators [31] have previously found very high levels of CDDP-DNA adducts to occur in tubular cells within 1 h of CDDP treatment in rodents, but very low staining intensity in glomerular cells. Thus, it seems plausible that the acute tubular damage is mediated by a rapid formation of CDDP-DNA adducts, whereas the glomerular damage occurs via other mechanisms.

The present study was designed to investigate the acute nephrotoxicity occurring after the first CDDP treatment. One pertinent question is whether early proteinuria has any clinical relevance. Most patients (eight out of ten) had elevated levels of protein HC at 20 h. By 3 weeks, two patients had a persistent, substantial elevation of protein HC (>3 mg/mmol), and those two patients were the only ones who developed clinically relevant renal dysfunction during the observation period. Thus, it is possible that patients who have not repaired their tubular damage within 3 weeks are at risk, but to elucidate the clinical significance of early proteinuria, more patients followed for a longer period are needed.

Correlations were found between the pharmacokinetics of total Pt in plasma and kidney damage, in accordance with the results of previous studies [4, 8]. Pt clearance correlated significantly with the level of protein HC at 20 h and C_{max}, with the level of protein HC at 3 weeks. Our study also suggests that it might be possible to identify threshold levels of (e.g.) AUC above which the risk of developing tubular kidney damage increases. Such thresholds may be clinically useful as predictive tools, but determination of those levels requires a number of patients larger than that investigated in the present study. No significant correlation between Pt pharmacokinetics and the levels of the glomerular damage markers albumin and IgG was observed. This suggests that it is mainly the tubular damage that is dependent on Pt pharmacokinetics, with low drug clearance being a risk factor for early

tubular injury and peak concentrations, for persistent tubular damage. Contrary to what might have been expected, we did not find any correlation between nephrotoxicity and CDDP-DNA adducts in normal cells. This may suggest that the adduct kinetics in kidney cells differs from that in lymphocytes and buccal cells.

Tumor response has been reported to correlate with total [8] and free [27] plasma Pt and with Pt-DNA adducts in blood leukocytes [5, 26, 27] and buccal cells [3]. In the present study there was no obvious relationship between tumor response and normal cell adducts, but we did find that all patients with a C_{max} of $> 2.7\ \mu g/ml$ or an AUC value of $> 300\ mg\,h\ l^{-1}$ responded to the therapy and that the two nonresponders had C_{max} and AUC values below those levels. However, it must be emphasized that this observation does not allow us to draw any firm conclusion due to uncertainties in the response estimation and to the low number of patients involved.

In summary, we found limited sampling for the analysis of total plasma Pt and a pharmacokinetic population model to be a useful approach for the comparison of pharmacokinetic parameters in a group of cancer patients after their first cycle of CDDP-containing chemotherapy. 'Older patients had a lower Pt clearance and more extensive early tubular kidney damage. Two normal cell types with different rates of turnover appeared to show different kinetic profiles for CDDP-DNA adducts. Analysis of the urinary excretion of marker proteins demonstrated that the nephrotoxic effects of CDDP are displayed first at the tubular level and later at the glomerular level. There were indications that tubular nephrotoxicity might be predicted by the pharmacokinetics of total plasma Pt. However, this and other correlations between pharmacokinetic parameters and toxicity as well as antitumor activity have to be confirmed in larger clinical studies. Efforts should also be put into defining pharmacodynamic threshold levels that, together with a limited-sampling approach for the analysis of CDDP pharmacokinetics at an early stage of chemotherapy, may provide the possibility of making individual dose adjustments, thereby optimizing the antitumor efficacy and preventing the development of serious side effects.

Acknowledgements Many thanks to Drs. L. den Engelse and F.A. Blommaert, Division of Molecular Carcinogenesis, the Netherlands Cancer Institute, Amsterdam, for providing us with the primary antiserum NKI-A59 and to Mrs. M. Nilsson at the Department of Occupational Medicine, University Hospital of Lund, for the FAAS analyses. This work was supported by grants from the Swedish Cancer Society, the John and Augusta Persson Society, and the foundations of the University Hospital of Lund.

References

1. Beal SL, Sheiner LB (1980) The NONMEM system. Am Stat 34 : 118
2. Beal SL, Sheiner LB (1982) Estimating population kinetics. CRC Crit Rev Biomed Eng 8 : 195
3. Blommaert FA, Michael C, Terheggen PMAB, Muggia FM, Kortes V, Schornagel JH, Hart AAM, Engelse L den (1993) Drug-induced DNA modification in buccal cells of cancer patients receiving carboplatin and cisplatin combination chemotherapy, as determined by an immunocytochemical method: interindividual variation and correlation with disease response. Cancer Res 53 : 5669
4. Campbell AB, Kalman SM, Jacobs C (1983) Plasma platinum levels: relationship to cisplatin dose and nephrotoxicity. Cancer Treat Rep 67 : 169
5. Dabholkar M, Bradshaw L, Parker RJ, Gill I, Bostick -Bruton F, Muggia FM, Reed E (1992) Cisplatin-DNA damage and repair in peripheral blood leukocytes in vivo and in vitro. Environ Health Perspect 98 : 53
6. Daley-Yates PT, McBrien DCH (1984) Cisplatin metabolites in plasma, a study of their pharmacokinetics and importance in the nephrotoxic and antitumour activity of cisplatin. Biochem Pharmacol 33 : 3063
7. Daugaard G, Rossing N, Rørth M (1988) Effects of cisplatin on different measures of glomerular function in the human kidney with special emphasis on high dose. Cancer Chemother Pharmacol 21 : 163
8. Desoize B, Marechal F, Millart H, Cattan A (1991) Correlation of clinical pharmacokinetic parameters of cisplatin with efficacy and toxicity. Biomed Pharmacother 45 : 203
9. Desoize B, Dumont P, Manot PL, Urien S, Dufour R, Berthiot G (1994) Comparison of two dose prediction models for cisplatin. Anticancer Res 14 : 2285
10. Fichtinger-Schepman AMJ, Oosterom AT van, Lohman PHM, Berends F (1987) *cis*-Diamminedichloroplatinum(II)-induced DNA adducts in peripheral leukocytes from seven cancer patients: quantitative immunochemical detection of the adduct induction and removal after a single dose of *cis*-diammine-dichloroplatinum(II). Cancer Res 47 : 3000
11. Fichtinger-Schepman AMJ, Velde-Visser SD van der, Dijk-Knijnenburg WCM van, Oosterom AT van der, Baan RA, Berends F (1990) Kinetics of the formation and removal of cisplatin-DNA adducts in blood cells and tumor tissues of cancer patients receiving chemotherapy: comparison with in vitro adduct formation. Cancer Res 50 : 7887
12. Fish RG, Shelley MD, Badman J, Mason M, Paterson I (1994) Platinum accumulation in adult cancer patients receiving cisplatin. Plasma and urinary platinum concentrations on first drug exposure. Drug Invest 7 : 175
13. Gamelin E, Allain P, Maillart P, Turcant A, Delva R, Lortholary A, Larra F (1995) Long-term pharmacokinetic behavior of platinum after cisplatin administration. Cancer Chemother Pharmacol 37 : 97
14. Grubb A (1992) Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 38 : S20
15. Grubb A (1992) Zur empfindlichen und schnellen Klassifizierungen der Proteinurie: Analyse einzelner Proteine im Urine. Diagn Lab 42 : 157
16. Hofmann W, Rossmüller B, Guder WG, Edel HH (1992) A new strategy for characterizing proteinuria and haematuria from single pattern of defined proteins in urine. J Clin Chem Clin Biochem 30 : 707
17. Johnsson A, Olsson C, Andersson H, Cavallin-Ståhl E (1994) Evaluation of a method for quantitative immunohistochemical analysis of cisplatin-DNA adducts in tissues from nude mice. Cytometry 17 : 142
18. Johnsson A, Olsson C, Nygren O, Nilsson M, Seiving B, Cavallin-Ståhl E (1995) Pharmacokinetics and tissue distribution in nude mice: platinum levels and cisplatin-DNA adducts. Cancer Chemother Pharmacol 37 : 23
19. Kelland LR (1994) The molecular basis of cisplatin sensitivity/resistance. Eur J Cancer 30A : 725

20. Kelsen DP, Alcock N, Young CW (1985) Cisplatin nephrotoxicity. Correlation with plasma platinum concentrations. Am J Clin Oncol 8 : 77
21. Leone R, Fracasso ME, Soresi E, Cimino G, Tedeschi M, Castoldi D, Monzani V, Colombi L, Usari T, Bernareggi A (1992) Influence of glutathione administration on the disposition of free and total platinum in patients after administration of cisplatin. Cancer Chemother Pharmacol 29 : 385
22. Manaka RC, Wolf W (1978) Distribution of cisplatin in blood. Chem Biol Interact 22 : 353
23. Patton TF, Repta AJ, Sternson LA, Belt RJ (1982) Pharmacokinetics of intact cisplatin in plasma. Infusion versus bolus dosing. Int J Pharm 10 : 7
24. Pinzani V, Bressolle F, Haug IJ, Galtier M, Blayac JP, Balmès P (1994) Cisplatin-induced renal toxicity and toxicity-modulating strategies: a review. Cancer Chemother Pharmacol 35 : 1
25. Reece PA, Stafford I, Russell J, Khan M, Gill PG (1987) Creatinine clearance as a predictor of ultrafiltrable platinum disposition in cancer patients treated with cisplatin: relationship between peak ultrafiltrable platinum plasma levels and nephrotoxicity. J Clin Oncol 5 : 304
26. Reed E, Ostchega Y, Steinberg SM, Yuspa SH, Young RC, Ozols RF, Poirier MC (1990) Evaluation of platinum-DNA adduct levels relative to known prognostic variables in a cohort of ovarian cancer patients. Cancer Res 50 : 2256
27. Schellens JHM, Ma J, Planting AST, Burg MEL van der, Schmitz PIM, Stoter G, Verweij J (1995) Exposure to cisplatin and WBC DNA-adduct formation determine the likelihood of response in solid tumor patients, which enables adaptive intrapatient dose-escalation. Proceedings, 7th international symposium on platinum and other metal coordination compounds in cancer chemotherapy, Amsterdam, March 1–4, 1995, p 55
28. Stewart DJ, Benjamin RS, Zimmerman S, Caprioli R, Wallace S, Chuang V, Calvo III D, Samuels M, Bonura J, Li Loo T (1983) Clinical pharmacology of intraarterial *cis*-diamminedichloroplatinum(II). Cancer Res 43 : 917
29. Sunderman FW Jr, Sporn J, Hopfer SM, Sweeney KR, Chakraborty NG, Greenberg B (1990) Platinum in blood mononuclear cells from patients after cisplatin therapy. Ann Clin Lab Sci 20 : 379
30. Tencer J, Thysell H, Andersson K, Grubb A (1994) Stability of albumin, protein HC, immunoglobulin G, kappa- and lambda-chain immunoreactivity, orosomucoid and alpha 1-antitrypsin in urine stored at various conditions. Scand J Clin Lab Invest 54 : 199
31. Terheggen PMAB, Floot BGJ, Scherer E, Begg AC, Fichtinger-Schepman AMJ, Engelse L den (1987) Immunocytochemical detection of interaction products of *cis*-diammindichloroplatinum(II) and *cis*-diammine (1,1-cyclobutanedicarboxylato) platinum(II) with DNA in rodent tissues. Cancer Res 47 : 6719
32. Terheggen PMAB, Dijkman R, Begg AC, Dubbelman R, Floot BGJ, Hart AAM, Engelse L den (1988) Monitoring of interaction products of *cis*-diammindichloroplatinum(II) and *cis*-diammine (1,1-cyclobutanedicarboxylato)platinum(II) with DNA in cells from platinum-treated patients. Cancer Res 48 : 5597
33. Vermorken JB, Vijgh WJF van der, Klein I, Hart AAM, Gall HE, Pinedo HM (1984) Pharmacokinetics of free and total platinum species after short-term infusion of cisplatin. Cancer Treat Rep 68 : 505
34. Vermorken JB, Vijgh WJF van der, Klein I, Gall HE, Groeningen CJ van, Hart GAM, Pinedo HM (1986) Pharmacokinetics of free and total platinum species after rapid and prolonged infusions of cisplatin. Clin Pharmacol Ther 39 : 136