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Pharmacokinetics and tissue distribution of cisplatin in nude mice: platinum levels and cisplatin-DNA adducts

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Abstract The pharmacokinetics of platinum (Pt) and cisplatin (CDDP)-DNA adducts were studied in nude mice after single-dose CDDP treatments. Whole blood, serum, kidney, lever, testis, brain, and tumor were collected at different intervals after injection of CDP at different dose levels. Pt was measured with flameless atomic absorption spectrometry (FAAS) or adsorptive voltammetry (AdV) and CDDP-DNA adducts with quantitative immunohistochemistry. The drug was immediately absorbed into the blood circulation (peak serum Pt levels were reached within 5 min) after i.p. CDDP administration, and distribution into most tissues also occurred rapidly (tissue Pt levels peaked at 15 min). With a sampling period of 7 days there was a biphasic elimination of Pt from blood, serum, and tissues. In the brain the pharmacokinetics differed with a gradual accumulation of Pt occurring during the 1st week. Formation of CDDP-DNA adducts in tissues was a slower process, with maximal levels being achieved at between 30 min and 4 h after drug administration, followed by a steady state lasting for at least 24 h. Each tissue type had its specific immunohistochemical staining pattern of adducts. With escalating CDDP doses there was a linear, or almost linear, increase in Pt concentrations and CDDP-DNA adduct levels in all sample types examined. These results suggest that a fair estimation of the amount of drug in tumor and normal tissues can be made from analysis of serum Pt at a fixed time point after a single dose of CDDP.

Key words Cisplatin · Pharmacokinetics · Tissue distribution · Cisplatin-DNA adducts

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Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] is a commonly used chemotherapeutic drug with high activity against several tumor types, such as testicular and ovarian cancer. Dosage of CDDP is calculated by the body surface of the patient, which is a rather crude way of determining the dose for individual patients. Knowledge on how the tissue distribution of CDDP correlates with the tumoricidal effect and with side-effects remains limited. With a better understanding of drug distribution and its clinical implications a more individualized chemotherapy should be possible in the future.

For quantitation of CDDP, several methods are available. Radiographic techniques can be used for analysis of radiolabeled platinum (Pt) [17, 19, 42]. Pt concentration can also be measured with different metal-detection methods, including X-ray fluorescence [1, 16], proton-induced X-ray emission (PIXE) [24], flameless atomic absorption spectrophotometry (FAAS) [40], inductively coupled plasma-mass spectrometry (ICP-MS) [25], and adsorptive voltametry (AdV) [27]. The latter method is very sensitive but fairly new, and the experience with Pt quantitation in tissues using AdV is limited.

The antitumor activity of cisplatin is thought to be mediated by its binding to DNA, leading to the formation of adducts and cross-links that lead to inhibition of DNA synthesis. Detection of CDDP in its probable target position can be performed by using different antisera elicited against the CDDP-DNA adducts. Quantitation of the adducts can be done by the use of

either enzyme-linked immunosorbent assay (ELISA) [11, 28] or immunohistochemistry [15, 36].

Analysis of CDDP in its target position bound to DNA may be biologically more relevant than Pt measurements in plasma or tissue homogenates. Pharmacokinetic investigations on the correlations between Pt analyses and CDDP-DNA adducts are lacking in the literature. The object of this study was to describe and compare the pharmacokinetics of CDDP in terms of Pt levels and CDDP-DNA adducts occurring in various tissues at different doses and intervals after i.p. CDDP treatment of nude mice.

Materials and methods

Study design

Time-dependent pharmacokinetics were studied in experiments in which nude mice were treated with a fixed CDDP dose and sampling was done at different intervals after injection. In the dose-dependence studies, sampling was done at a given time point after injection with escalating CDDP doses. Pt concentrations were measured in whole blood and serum. In kidney, liver, testis, brain, and tumor, Pt content as well as CDDP-DNA adducts were analyzed. Pt levels were measured with AdV for time-dependent pharmacokinetics and with FAAS for dose-dependent pharmacokinetics. There were practical reasons for this: the two studies were performed during slightly different periods, during which we had access to either AdV or FAAS. CDDP-DNA adducts were analyzed with quantitative immunohistochemistry.

In vivo model

Nude BALB/c mice (nu/nu) with a xenografted squamous-cell carcinoma tumor line (AB) were used. This in vivo model has been characterized by Wennerberg et al. [41], who reported a dose-dependent retardation of tumor growth. A CDDP dose of 7.5 mg/kg or higher resulted in a statistically significant inhibition of tumor growth. CDDP doses of 7.5–10 mg/kg were estimated to correspond to the dose lethal to 10%–20% of the mice (LD_{10-20}) [41], whereas further increases in the dose resulted in enhanced mortality without any corresponding increase in the tumoricidal effect. Therefore, the CDDP dose of 7.5 mg/kg was chosen for the time-dependent study.

Treatment and tissue preparation

Male nude mice aged 5–8 weeks were inoculated s.c. with the tumor cell line AB in its 120th passage, 2 tumors on each animal. At the time of the experiments, approximately 3 weeks after transplantation, the tumors were 7–12 mm in diameter. CDDP (Bristol-Myers-Squibb; 0.5 mg/ml) diluted in NaCl (9 mg/ml) was given i.p. as rapid bolus injections. The experiments were approved by the Ethics Committee for Animal Studies at the University of Lund.

In the time-dependence studies the mice received 7.5 mg/kg CDDP and were killed at the following time points: 0 (untreated controls), 5, 10, 15, 20, and 30 min and 1, 4, 12, 24, and 168 h after treatment, with 3–5 animals being killed at each time point. The mice were anesthetized with ether and exterminated by bleeding. Half of the blood volume was heparinized and saved as whole blood and the rest was first allowed to clot at room temperature and then centrifuged at 1500 rpm for 10 min to obtain serum. Whole blood and serum were frozen at − 70°C for later Pt analysis. Tissues were quickly removed and divided into two portions. One of the pieces was frozen directly at − 70°C for later Pt analysis. The other tissue piece was frozen on dry ice, after which multiple 10-µm cryostat sections were prepared on poly-L-lysine-coated slides and stored at − 70°C for later CDDP-DNA analysis. For the dose-dependence experiments, mice were injected with 0, 3.75, 7.5, or 15 mg/kg CDDP, with three animals being treated at each dose level. Blood, serum, and tissues were collected at 1 h after administration and prepared as described above. The reason for choosing this sampling time point was that pilot experiments had indicated that the Pt concentrations in serum as well as in tissues reached a steady state at approximately 1 h after CDDP administration.

Tumor volume measurements

On the mice that were to be analyzed at 168 h after CDDP treatment, tumors were measured before treatment and after 1 week. Two orthogonal diameters were measured with vernier calipers. The tumor volume was calculated as follows [41]:

$$\text{Volume} = (\text{length} \times \text{width}^2)/2 .$$

Adsorptive voltammetry

The AdV analyses were performed as described previously [27], with the following modifications. Two aliquots (10–50 µl) of each thawed blood and serum sample were pipetted into silica crucibles (Suprasil; Werner Glas AB, Stockholm, Sweden) and two pieces (5–50 mg) of each thawed tissue sample were weighed into crucibles. A Pt nitrate addition was made to one of each sample replicate. To all crucibles, 25 µl HNO_3 was added and the samples were ashed at 800°C [27]. After careful evaporation of the final acid ashing solution, 600 µl hydrochloric acid was added. After 10 min, 2.2 ml water was added to dissolve the dried sample and the solution was transferred into a 10-ml screw-capped glass vial. The crucible was rinsed with 2.2 ml water, which was transferred to the vial, to obtain a final volume of 5 ml. An aliquot of the sample (10–1000 µl) was pipetted into the voltammetric cell and the analytical reagents were added [27]. The sample was stirred and deaerated for 5 min and then, depending on the Pt concentration, preelectrolyzed for 20–120 s at − 800 mV versus the reference electrode. After a 10-s quiescent period, the stripping was recorded and the Pt content was quantified [27]. The mean value obtained for the two replicates after subtraction of the initial addition was calculated and used as a measure of the Pt concentration in the sample. If the difference between the sample replicates was larger than 15%, the sample was reanalyzed. The detection limit has previously been determined to be 0.17 ng/ml in a 100-µl sample [27].

Flameless atomic absorption spectrophotometry

Samples were thawed at room temperature. Blood and serum specimens (70–200 µl) were diluted at least 10-fold in matrix modifier (2 mg NH_4NO_3/ml deionized water with 0.2% Triton-X 100) before analysis. Tissue samples (15–450 mg) were digested overnight with concentrated HNO_3 in screw-capped plastic tubes at 105°C. Then, samples were incubated with H_2O_2 for 1 h at 90°C for further oxidation and background reduction. Deionized water was added to a fixed volume. All samples were analyzed in a Perkin-Elmer 5000 AA device equipped with deuterium background correction, an HGA 500 graphite furnace, and an AS 40 autosampler. Atomization was performed from the walls of pyrolytic graphite tubes. The inert gas was argon. The instrument was programmed for drying at 120°C, ashing at 1500°C, and atomization at 2600°C with maximal power heating and gas stop. Each specimen was analyzed twice and

the mean value was calculated. The Pt concentrations were determined by a standard addition procedure for each sample. The detection limit for blood and serum was approximately 50 ng/ml in a 100-µl sample.

CDDP-DNA adduct analysis

The immunostaining protocol of Terheggen et al. [36] was used, with some modifications as previously described [15]. The general outlines were as follows. Each staining batch consisted of a maximum of 19 slides. The slides were treated with H_2O_2 (to inactivate endogenous peroxidase) and NaOH (to denature DNA) before incubation with anti-CDDP-DNA rabbit antiserum NKI-A59 (a gift from Prof. Leo den Engelse, Netherlands Cancer Institute, Amsterdam) diluted 1:2000 in phosphate-buffered saline (PBS) with fetal calf serum and calf-thymus DNA. Slides were then sequentially incubated with GAR (goat anti-rabbit immunoglobulin; Dakopatts, Copenhagen, Denmark) diluted 1:600 and PAP (peroxidase antiperoxidase complex; American Qualex, La Mirada, Calif., USA) diluted 1:3000. The GAR and PAP steps were repeated once. A brown staining reaction was developed with diamminobenzidine, and the slides were finally counterstained with methyl green.

Quantitation of the CDDP-DNA adducts was performed with the computer-assisted image analyzer CAS 200 (Cell Analysis System, Elmhurst, Ill. USA) using a two-color mask-image technique [4]. The quantitative nuclear antigen (QNA) software package was utilized. This image-analysis procedure has been thoroughly described and evaluated in a previous report [15]. All slides in the present study were measured by the same observer. As each tissue type has its specific staining pattern with differentiated adduct formation in different histological structures [15], the topographic areas of interest were decided for each organ type as presented in Results. On each slide, 15–25 representative 40x fields with a minimum of tissue folds and other artifacts were measured. A total of 700–2000 cells/slide were thus analyzed. For each field, the CAS computer calculates the "positive area," which represents the percentage of positive nuclear area (PNA). The PNA values obtained for each single field are merged to produce mean values for each slide. To adjust for nonspecific nuclear staining, slides from two or three untreated control animals were included in each staining batch. The PNA values of the controls were then subtracted from the remaining slides of that batch. This method should be used for comparisons of slides prepared from the same tissue type, stained in the same batch, and preferably measured by one observer at one occassion [15].

Pharmacokinetic calculations

Pharmacokinetic parameters were calculated according to a compartment-independent approach. The elimination rate constants were estimated from the slope of each part of the log concentration-time profiles. A minimum of four data points were employed. Data analysis was performed with SIPHAR computer software (Simed, Créteil, France).

Results

Time-dependent pharmacokinetics

Platinum concentrations and elimination

The pharmacokinetic parameters are presented in Table 1 and the time-concentration profiles are shown in Figs. 1a and 1b. Absorption of the i.p.-injected CDDP occurred very rapidly, and the peak Pt concentration in whole blood and serum was reached within 5 min. The peak Pt concentration in kidney and liver was observed at 15 min, with the peak in kidney being almost twice as high as that in liver. The testis, brain, and tumor tissues were not examined with regard to early Pt distribution. In testis and tumor, maximal Pt concentrations were observed in the first sample at 30 min posttreatment. In the brain there was a very low initial Pt level followed by a gradual accumulation during the 1st week. It could not be determined whether the peak level was reached within 168 h. Tissue/serum ratios for the Pt concentrations increased gradually with time in all tissues (Table 2).

A sampling period of 7 days gave a biphasic elimination pattern of Pt from whole blood, serum, and all tissues except brain. There was one initial elimination phase from blood, serum, and tissues during the 1st h ($t_{1/2\ initial}$, 9–30 min; Table 1), followed by a slow decline of Pt concentrations in a terminal phase over the next 7 days. The terminal half-lives were similar in whole

Table 1 Pharmacokinetic parameters obtained for whole blood, serum, and tissues from nude mice after i.p. injection of 7.5 mg/kg CDDP. Platinum was measured with ADV (T_{max} time for maximal concentration, C_{max} maximal concentration, $t_{1/2\ initial}$ initial half-life, $t_{1/2\ terminal}$ terminal half-life, $AUC_{0.5-168h}$ area under the concentration-time curve for the interval of 0.5–168 h, *ND* not determined)

Sample	T_{max} (min)	C_{max} [a]	$t_{1/2\,initial}$ (min)	$t_{1/2\,terminal}$ (h)	$AUC_{0.5-168h}$ [b]
Blood	5	12.2	15	58	75.4
Serum	5	13.6	9	55	16.0
Kidney	15	24.2	23	51	290.7
Liver	15	13.0	30	76	444.7
Tumor	30[c]	3.0	*ND*	173	155.4
Testis	30[c]	1.1	*ND*	64	56.5

[a] µg/ml in blood and serum, µg/g in tissues
[b] µg h ml^{-1} in blood and serum, µg h g^{-1} in tissues
[c] Not examined prior to 30 min

Table 2 Tissue/serum ratios (mean values) of Pt concentrations measured in tumor-bearing nude mice at different time points after i.p. injection of 7.5 mg/kg CDDP (*ND* Not determined)

Time	Tissue/serum ratio (µg/g:µg/ml)				
	Kidney	Liver	Testis	Brain	Tumor
5 min	1.6	0.6	ND	ND	ND
10 min	1.7	0.7	ND	ND	ND
15 min	2.9	1.5	ND	ND	ND
20 min	5.1	3.2	ND	ND	ND
30 min	7.2	4.9	1.0	0.2	2.8
1 h	10.7	8.3	1.4	0.8	1.4
4 h	15.5	15.5	1.8	2.6	4.9
12 h	19.4	18.3	2.5	2.9	3.7
24 h	20.9	33.8	4.7	7.6	9.7
168 h	16.4	40.9	4.5	53.7	29.4

blood, serum, kidney, liver, and testis ($t_{1/2\,\text{terminal}}$, 51–76 h), whereas the terminal half-life in tumor was 173 h. In whole blood there was a second Pt peak at 24 h (Fig. 1a). Since this was an unexpected finding, a complementary study of animals sampled at 12 and 24 h was performed, in which the second peak was confirmed (data not shown).

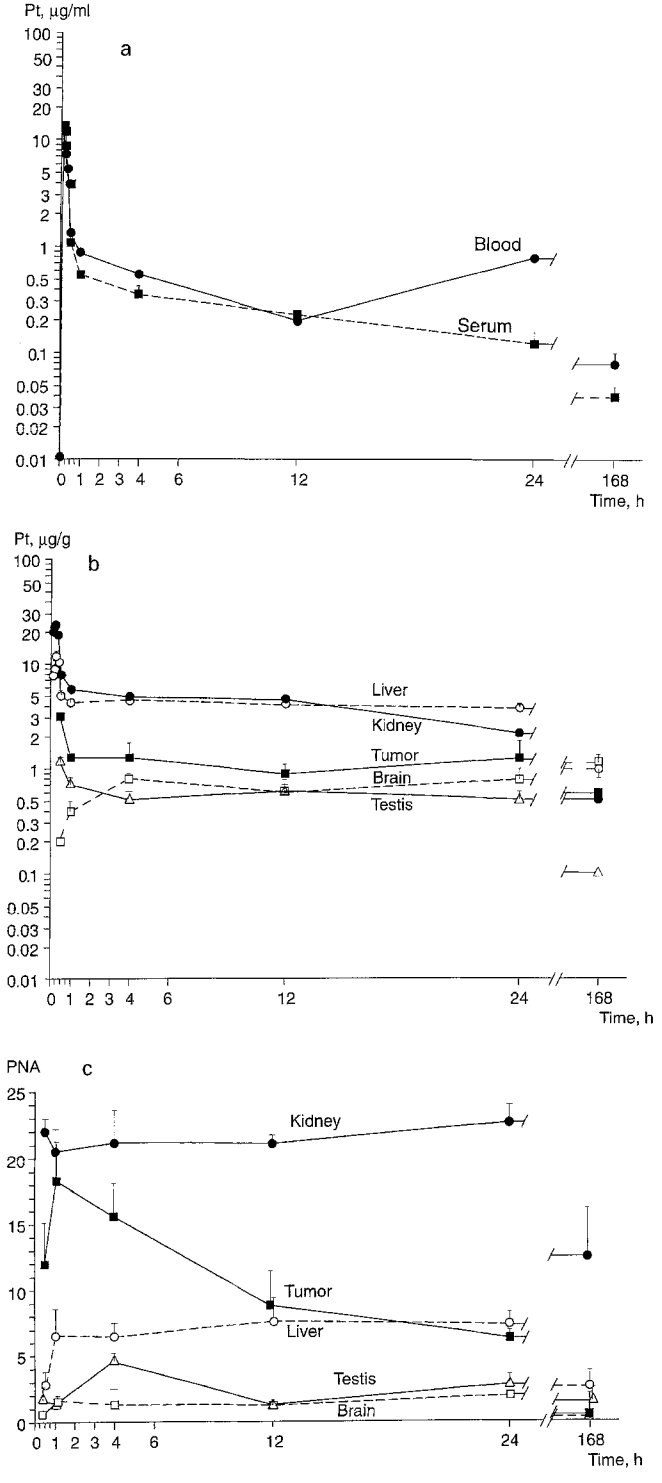

Fig. 1a–c Time-dependent pharmacokinetic profiles obtained after single i.p. doses of 7.5 mg CDDP/kg in tumor-bearing nude mice. Data represent mean values ± SE. **a** Pt concentrations in whole blood and serum. **b** Pt concentrations in tissues. **c** CDDP-DNA adducts expressed as PNA (percentage of positive nuclear area) in tissues (not for interorgan comparisons of PNA levels)

The areas under the concentration-time curve (AUC) were calculated for the intervals of 0–168 as well as 0.5–168 h and the results were very similar. To allow inclusion of testis and brain, in which the $AUC_{0-168\,h}$ could not be calculated, only the $AUC_{0.5-168\,h}$ values were used for analysis (Table 1). The AUC was 5 times greater in whole blood than in serum due to the 24-h peak in blood. Regarding the tissues, the liver had the greatest AUC, which was approximately 1.5-fold that in the kidney, 3-fold that in the tumor, and 8-fold that in the testis.

Cisplatin-DNA adducts

The immunohistochemical staining patterns of CDDP-DNA adducts in different tissues have previously been described [15, 36]. The staining patterns did not alter with the CDDP dose or with the interval after injection. In the *kidney* there was strong nuclear staining in the cortex, especially in some tubular structures, and weaker staining in the medulla. In this study the outer cortical areas of the kidney were measured. The adduct distribution in the *liver* was fairly homogenous over different lobuli, mostly appearing in the nuclei of the hepatocytes. In the *testis*, CDDP-DNA adducts were seen exclusively in interstitial cells, whereas no staining was found in the germ cells. Only interstitial cells were measured. As the positively stained interstitial cells were rather few and scattered in groups, the field-selection process was difficult, which could have affected the reliability of the results. In the *brain* there was a fairly weak staining in neuronal as well as glial types of cells. The way in which the sections were prepared did not allow the deeper structures of the brain to be studied in a standardized way. In this study only the cortical parts of the brains were analyzed for CDDP-DNA adducts. The overall low staining intensity along with some nonspecific background staining could have affected the reproducibility of the image analysis negatively. The results of the adduct analyses of the brain should therefore be interpreted with some caution. In *tumors* there was a relatively weak staining intensity in tumor cells, gathered in nodules. In most tumors there were regions of stromal cells with strong staining. Many tumors had areas of necrosis. In necrotic and "perinecrotic" cells there was often very strong staining. In this study the staining intensity was measured in the nuclei of morphologically intact and, presumably, vital tumor cells only.

The kinetic profiles of the CDDP-DNA adducts (Fig. 1c) are described for the period ranging from 30 min to 168 h. Prior to 30 min the adducts were not examined in a consistent way. However, a few kidneys and livers from animals exterminated at 10–20 min

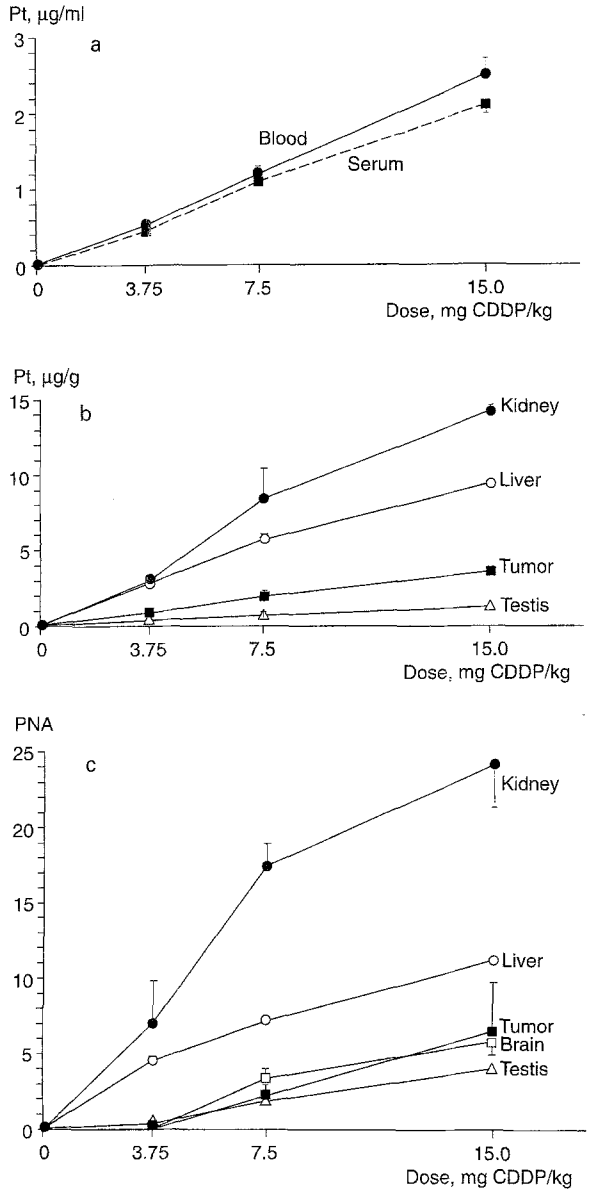

Fig. 2a–c Dose-dependent pharmakokinetic profiles obtained following single i.p. doses of CDDP in tumor-bearing nude mice. Samples were collected at 1h postinjection. Data represent mean values ± SE. **a** Pt concentrations in whole blood and serum. **b** Pt concentrations in tissues. **c** CDDP-DNA adducts expressed as PNA (percentage of positive nuclear area) in tissues (not for interorgan comparisons of PNA levels)

after injection were examined by subjective microscopic evaluation, and the intensity of the immunohistochemical staining was clearly lower than that seen in the ones from the animals killed at 30 min, implying that there was no adduct peak prior to 30 min postinjection.

The maximal adduct level, expressed as PNA, was reached at 30 min in the kidney; at 1 h in the liver, brain, and tumor; and at 4 h in the testis (Fig. 1c). In the kidney and liver and, possibly, also in the testis and brain there was then a steady state until at least 24 h. In tumors there was a gradual decrease in adduct levels,

Table 3 Tissue/serum ratios (mean values) of Pt concentrations measured in tumor-bearing nude mice at 1 h after i.p. injection of escalating CDDP doses

Dose (mg/kg)	Tissue/serum ratio (µg/g:µg/ml)			
	Kidney	Liver	Testis	Tumor
3.75	6.8	5.7	0.7	1.8
7.5	7.5	5.2	0.6	1.8
15	6.8	4.5	0.6	1.6

beginning after 1 h. By 168 h a considerable elimination of adducts had occurred in all tissue types.

Dose-dependent pharmacokinetics

Platinum concentrations

In whole blood and serum there was a linear correlation between the CDDP dose and the Pt concentration, with only minor variations occurring within each dose level (Fig. 2a). In the organs there were also good correlations between dose and Pt concentration (Fig. 2b). The Pt levels obtained in the dose-dependence study were analyzed with FAAS, and all the brain samples were below the detection limit for the method. The tissue/serum ratios did not change with increasing CDDP dose (Table 3).

CDDP-DNA adducts

With escalating CDDP doses there were increasing CDDP-DNA adduct levels in all tissues (Fig. 2c). The interindividual variations were fairly large, especially for the kidney and tumor at the highest CDDP dose (15 mg/kg). At the lowest dose of 3.75 mg/kg the adduct formation was below the detection limit in testis, brain, and tumor tissues.

Toxicity and tumor response

Most animals were killed as early as within 24h of CDDP treatment and were thus not evaluable for toxicity or tumor response. However, no toxic death was observed. In the five mice in the time-dependence study that were followed for 168h after CDDP treatment, changes in body weight and tumor-growth delay were analyzed. There was a mean decrease in body weight of 16% (range, 0–33%). The five mice had a total of eight measurable tumors and there was an average volume reduction of 15% ($P = 0.08$, paired t-test) in a comparison of tumor sizes measured before and at 168 h after treatment.

Discussion

This study describes the pharmacokinetic relations between the total platinum levels measured in serum, whole blood, and tissues versus the cisplatin-DNA adducts detected in tissues from nude mice. Two different methods were used for Pt analysis. FAAS can be regarded as a standard method for Pt quantitation in various types of biological samples, whereas AdV is a fairly new method. Although the experience with AdV has thus far been limited, this technique has recently been employed for determination of Pt in biological samples [27]. The advantage of AdV is its low detection limit, which was exemplified in the present study by the measurements obtained in brain tissue. The AdV measurements gave detectable Pt levels, whereas no Pt could be traced with FAAS despite escalating CDDP doses.

The biphasic elimination of total Pt from serum, involving initial and terminal Pt half-lives, was well in accordance with previous reports on rodents [10, 22, 38, 42] and patients [35, 39]. In the systemic circulation, Pt levels were analyzed not only in serum but also in whole blood, which has not previously been used for studies on CDDP pharmacokinetics, and the interpretation of the whole-blood profile is not evident. The main quantitative difference between whole blood and serum values are the erythrocytes. Thus, it is plausible that the second peak observed in whole blood is an indirect way of viewing a second peak in red blood cells. Second peaks of Pt in erythrocytes have not been described previously, and the mechanism for the second peak observed in the present study is unclear. It might be caused by redistribution of the drug from deeper compartments into the circulation and increased binding of the drug to erythrocytes with impaired membrane function due to earlier drug exposure. Binding of CDDP to erythrocytes could be of clinical relevance as a possible explanation for hemolytic anemias that sometimes occur after CDDP treatment of patients [20, 40]. Further studies are needed for clarification.

In the present study the Pt pharmacokinetics were also calculated in tissues. Analysis of tissue Pt concentrations have been presented in several previous reports [6, 14, 17, 21, 22, 24]. Our study also includes calculations of elimination half-lives and AUC values, which to our knowledge have not been described before. The time profiles and the elimination parameters obtained in tissues were similar to those recorded for serum and whole blood, with some exceptions. There was a delayed terminal elimination of Pt in tumors as compared with the other tissues (Table 1), which is discussed below. In the brain a peak Pt concentration was not reached during the study period of 7 days and, consequently, no pharmacokinetic parameter could be calculated. With a longer observation period this Pt accumulation in the brain probably would have added to a further prolongation of the terminal half-life in the central compartments as well such as serum. There was a pronounced and continuous increase in tissue/serum ratios for all tissue types examined (Table 2). The kidney/serum ratios found in the present study were very similar to the kidney/plasma ratios described by Fulco et al. [14]. These increasing tissue/serum ratios indicate a gradual retention of CDDP in tissues relative to serum.

The CDDP-DNA adducts were measured with an immunohistochemical method. Results obtained in immunohistochemical assays should be regarded as semi quantitative rather than quantitative. They all suffer in that the stoichiometric relations are incompletely known. With regard to the present study, we do not know if a doubled PNA (percentage of positive nuclear area) corresponds to twice as many adducts. However, the dose-dependence study showed that increased CDDP doses definitely gave stronger staining, and for most tissue types a doubled dose gave PNA values that increased approximately 2-fold. Each tissue type has its specific staining pattern [15], which influences the image-analysis results. Thus, interorgan comparisons of the PNA values (Figs. 1c, 2c) should be avoided. Moreover, sometimes a considerable variation in staining levels is observed between different batches [15]. In the present study the tumor PNA values obtained in the time-dependence study were coincidentally much higher than those obtained in the dose-dependence study (Figs. 1c, 2c) due to the samples having been stained in different batches. This emphasizes that the method should be used only for comparisons of slides from the same tissue type stained in one batch. Thus, in the present study conclusions should be drawn on the basis of only the shapes of the profiles, not the PNA levels.

Whereas the distribution of CDDP into the tissues expressed as Pt concentrations occurred rapidly (peak levels were achieved at 15 min, the formation of CDDP-DNA adducts was a slower process (peak levels were reached at between 30 min and 4 h). After approximately 1h the adduct and Pt profiles in most organs were basically parallel, indicating a steady state between the total amount of Pt compounds in the tissue and the proportion of CDDP bound to DNA. Similar observations were made by Fichtinger-Schepman et al. [12], who analyzed tissue Pt concentrations and DNA adducts in different tissues from CDDP-treated rats at two time points: 1 and 24 h after treatment.

The highest Pt concentrations measured over the first few hours after CDDP administration were found in the kidney, in agreement with several previous reports [6, 12, 14, 17, 21, 22, 24]. The highest levels of CDDP-DNA adducts were seen in the outer cortex, especially in some tubular structures. This finding has been described elsewhere [36]. Since the renal toxicity is at least in part believed to be of tubular origin [7, 43], the high adduct levels occurring in tubular structures

may be regarded as indirect evidence that the CDDP-DNA adducts observed with immunohistochemistry are of biological relevance.

Although the Pt levels detected in the liver were lower than those measured in the kidneys for the 1st day after CDDP administration, the long-term retention expressed as the AUC for the 1st week was greater in the liver than in the kidneys. This observation is in agreement with previous animal investigations [2, 6, 17, 21, 24] and autopsy studies on CDDP-treated patients [23, 37], in all of which long-term Pt levels were higher in the liver than in the kidney. Although high amounts of Pt are retained in the liver and CDDP-DNA adducts are formed at readily detectable levels, hepatotoxicity is rarely a clinical problem. One explanation on the cellular level might be that most hepatocytes are normally resting in the G0 phase, during which the cytotoxicity of CDDP is low. Another reason might be the large storage capacity of the liver or the efficient detoxification of substances, e.g., metallothioneins, that are produced in large amounts in the liver [33]. It might also indicate that high peak levels (as in the kidney) cause more damage to normal tissues than does long-term retention of CDDP.

The relatively low Pt levels found in the testis have been described before [42], probably reflecting the existence of a "blood-testis" barrier. The CDDP-DNA adducts were restricted to interstitial cells and were absent in germ cells, as has been described by Terheggen et al. [36]. This distribution pattern could at least in part explain why many patients treated with CDDP-containing chemotherapy regain their fertility within a few years after treatment.

The slow uptake of CDDP in the brain was possibly due to the blood-brain barrier. The Pt concentrations measured in the brain were fairly low, supporting previous reports [6, 19, 21, 22, 34, 42]. We did not observe the initial Pt peak seen in the other organs, but a gradual accumulation occurred during the 1st week. This suggests that the blood-brain barrier constitutes a barrier not only to influx but also to elimination of the drug. The Pt and adduct profiles differed in that there was no increase in adduct levels at 7 days. This finding should be interpreted with caution due to suboptimal technical conditions for adduct analysis, but it could imply that the retained Pt was not bound to nuclear DNA. One may speculate that an increasing portion of the Pt was bound to cerebral proteins. In contrast, Poirier et al, [29] reported high levels of Pt-DNA adducts in autopsy brain samples from patients at several months after CDDP therapy. One reason for this discrepancy might be differences in dosage; the patients in the aforementioned study [29] were given multiple CDDP treatments amounting to high cumulative doses, whereas our mice received only single doses of CDDP. Thus, studies on CDDP pharmacokinetics in brain tissue show somewhat conflicting results and further studies are needed to elucidate the mechanisms.

In tumor cells, correlations between cellular Pt concentration and sensitivity to CDDP are often found [3]. Decreased accumulation of Pt is a consistent finding in many CDDP-resistant cell lines. The tumor cell line ÅB has previously been proven to be sensitive to CDDP treatment [41]. Although the present study did not focus on antitumor activity, a moderate reduction in tumor volume was noticed in the animals kept alive for 1 week, which at least does not contradict that the cell line is CDDP-sensitive. Even so, tumor Pt concentrations were relatively low, considerably lower than the levels measured in the kidney or liver. This finding is in good agreement with many previous reports on sensitive as well as resistant tumors [6, 12, 14, 17, 42]. Assuming that the tissue concentration of a drug is of importance for the tumoricidal effect, this might be regarded a somewhat discouraging observation. Immunohistochemical analysis of CDDP-DNA adducts in tumor tissue has not been described before, and the biological relevance of the staining pattern is unknown. The time profile of CDDP-DNA adducts in apparently vital tumor cells showed a peak at 1h followed by gradually decreasing adduct levels, in contrast to the delayed terminal elimination of total Pt from the tumor tissue. The gradual decline of adducts could be explained by repair of damaged DNA. It could also reflect that cells with high initial levels of CDDP-DNA adducts died off. The prolonged retention of Pt indicates that an increasing portion of the "waste" Pt was accumulated in forms other than being bound to DNA in vital tumor cells, tentatively, in necrotic areas.

Pt concentrations and the levels of CDDP-DNA adducts increased with escalating CDDP doses, which has also been reported by other investigators [30, 31, 36]. The correlation was linear for serum and whole blood and almost linear for tissues. The tissue/serum ratios were independent of the CDDP dose in all tissue types. This indicates that with a serum Pt measurement made at a fixed time point after administration of a known dose of CDDP, the amount of drug in tumors and normal tissue may be estimated.

Experiments on mice are usually performed with CDDP doses in the range of 5–10 mg/kg, at which antitumor activity is seen and toxicity is acceptable [6, 14, 41]. That the dose-dependent pharmacokinetics did not reach a plateau within the dose range of 0–15 mg/kg suggests that the kinetics of CDDP are not saturated in the clinically feasible dose interval. Assuming a correlation between drug concentration in tissues and effect, tumoricidal effects as well as side effects would increase with escalating dose. In the clinical setting, escalation of the CDDP dose has indeed failed to improve the therapeutic index [26]. When the dose is raised above the "standard" range the toxicity tends to increase more than the tumoricidal effect.

What is the clinical relevance of these results obtained after the i.p. administration of single bolus doses to mice when patients are usually treated with i.v.

infusions? The pharmacokinetics of chemotherapeutic agents, including CDDP, are very similar in humans and nude mice [18]. CDDP administration by the i.p. route gives lower peak concentrations in plasma than does i.v. injection, but the respective terminal elimination and AUC values are similar [24]. Bolus injections give higher peak Pt levels and shorter initial half-lives in plasma than do infusions [40], but the terminal half-lives are independent of the dose schedule. Thus, Pt pharmacokinetics after i.p. bolus dosing and i.v. infusion differ mainly during the first period, i.e., 1–2 h after injection. This suggests that the results we obtained in nude mice may provide a fair estimate of the clinical pharmacokinetics – at least of what occurs after the initial period postinjection.

The aim of this study was to describe pharmacokinetic correlations in different tissue types. The effects on tumors and normal tissues were not analyzed. From the clinician's point of view, pharmacokinetics studies are of interest in that they increase the level of understanding, but more important are studies on correlations between pharmacokinetic and clinical parameters. Very few such studies on chemotherapeutic agents, including CDDP, have been undertaken. In CDDP-treated patients, increased levels of total plasma Pt have been correlated with nephrotoxicity [8] and tumor response [9]. High levels of CDDP-DNA adducts in white blood cells [13, 32] and buccal cells [5] from CDDP-treated patients have also been shown to correlate with tumor response. Further investigations on these clinicopharmacological correlations will hopefully provide us with predictive tools that can be used for designing a more individualized chemotherapy.

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