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Quantitative subcellular distribution of platinum in rat tissues following i.v. bolus and i.v. infusion of cisplatin*

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Summary. An analysis of the subcellular localization of platinum was conducted in Sprague-Dawley (SD) rats following administration of an i.v. dose of 6 mg/kg cisplatin (bolus and infusion). Biodistribution studies were carried out in the liver and kidney of control animals, as well as in these same organs and in the tumor (Walker 256 adenocarcinoma) of SD rats. The results obtained illustrate that in addition to the platination of DNA in these tissues, significant amounts of Pt are also incorporated into the chromosomal protein (CP) and cytosolic fractions. The localization of Pt in the cytosolic fractions was highest in the kidney, followed by the tumor, and lowest in the liver when determined as the fractional percentage of the total amount of injected drug (%ID/g). The significance of such cytosolic and CP localization of Pt is not known at this time, but they may be involved in the cytotoxic effects of cisplatin, as this drug is cytotoxic to tumors and kidneys but not to the liver. The localization of cisplatin in the subcellular fractions of liver, kidney, and tumor showed a trend toward being higher after i.v. infusion than after i.v. bolus administration of the drug.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] is an important drug for the treatment of a variety of human malignancies [6, 7, 19, 20, 23]. Its presumed mechanism of action is that it binds to either cell-surface DNA [22] or cross-link DNA through either inter- or intrastrand cross-links or DNA-protein cross-links [28]. Zwelling and Kahn [28] argued that DNA-protein cross-links were primarily associated with cytotoxicity rather than with anticancer activity.

Most of these prior studies focused on the cisplatin-DNA interactions and largely ignored the importance of cisplatin-protein (in the cytosol and nucleus) interactions that did not involve DNA or RNA. In addition, since most of the prior mechanistic studies involved cell cultures rather than animal in vivo systems, only limited data is available about the subcellular distributin of platinum (cisplatin) in vivo in animal tumor models. Reed et al. [21] studied cisplatin-DNA adduct formation in renal, gonadal, and tumor (sarcoma) tissues in Sprague-Dawley rats in vivo. They reported that renal tissue showed the highest cisplatin-DNA adduct formation, followed by the tumor and gonadal tissues. Schepman et al. [24] determined cisplatin-DNA adduct formation in vivo in rats bearing either cisplatin-sensitive or -resistant tumors (immunocytomas) and measured such adducts in rodent kidney, liver, tumor, and spleen tissues. Their study revealed that platinum levels were highest in the DNA of the kidneys, followed by that of the liver, tumor, and spleen, 1 h after cisplatin administration. In another study [9], the cellular concentration of platinum and total amounts of DNA cross-links and interstrand cross-links were determined in vitro in human melanoma cells and other cell lines. None of these investigators, however, measured the platinum content of other cellular fractions in addition to that of DNA.

We report the results of preliminary biodistribution studies in control and tumor-bearing rats after either an i.v. bolus or an infusion of cisplatin, illustrating that a significant amount of platinum appears to bind to the nucleoprotein fraction and to cytosolic ligands and suggesting that, in addition to the inhibition of DNA synthesis, such interactions with chromosomal proteins (histones, nonhistones, and protamines) and cytosolic ligands should also be considered in attempts to understand the mechanism of action of cisplatin.

Materials and methods

Animals. Male Sprague-Dawley rats weighing 250 g were used in all experiments. The rats were housed individually and were given free

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Table 1. Localization of cellular platinum in the tissues of tumor-bearing rats, expressed as the percentage of total platinum present in the tissue to the amount present in each fraction, 4 h after administration of an i. v. bolus dose of 6 mg/kg cisplatin ($n = 5$)

	DNA	Chromosomal proteins	Cytosolic ligands	
			LMW	HMW
Liver	9.48 ± 1.63	37.97 ± 11.34	20.62 ± 4.6	28.05 ± 9.0
Kidney	3.03 ± 1.2	22.77 ± 6.19	38.00 ± 7.41	34.11 ± 9.7
Tumor	2.23 ± 0.71	27.87 ± 7.28	29.68 ± 4.03	40.21 ± 8.5

LMW, low-molecular-weight cytosolic fraction; HMW, high-molecular-weight cytosolic fraction

Table 2. Localization of platinum in the subcellular fractions of liver, kidney, and tumor tissues 4 h after an i. v. bolus dose of 6 mg/kg cisplatin

	DNA	CP	LMW	HMW
Amount (ng × 10^2):				
Liver	5.81 ± 2.89	30.7 ± 12.5	16.8 ± 3.82	20.4 ± 5.1
Kidney	1.24 ± 3.49	1.13 ± 5.7	17.8 ± 8.02	15.9 ± 7.3
Tumor	0.54 ± 0.33	5.39 ± 2.1	5.56 ± 1.15	7.49 ± 1.7
%ID/organ (× 10^{-2}):				
Liver	4.59 ± 2.23	24.2 ± 10	13.1 ± 2.8	15.9 ± 3.7
Kidney	0.99 ± 0.25	8.79 ± 4.3	13.9 ± 5.9	12.7 ± 6.7
Tumor	0.43 ± 0.27	4.13 ± 1.7	4.35 ± 0.8	5.84 ± 1.1
%ID/g (× 10^{-2}):				
Liver	0.53 ± 0.26	2.78 ± 1.1	1.49 ± 0.3	1.82 ± 0.4
Kidney	0.68 ± 0.17	5.98 ± 2.9	9.41 ± 4	8.6 ± 4.5
Tumor	0.11 ± 0.07	1.06 ± 0.4	1.8 ± 0.3	2.42 ± 0.5

DNA fraction of kidney, liver and tumor, $N = 8$; other fractions, $n = 5$. LMW, low-molecular-weight cytosolic fraction; HMW, high-molecular-weight cytosolic fraction; CP, chromosomal protein fraction; %ID, fractional percentage of the total injected drug.
Tissue weights in grams: liver, 8.69 ± 0.68 ($n = 8$); kidney, 1.47 ± 0.07 ($n = 8$); tumor, 2.49 ± 0.92 ($n = 7$)

Table 3. Localization of platinum in the subcellular fractions of liver, kidney, and tumor tissues 4 h after a 1 h i. v. infusion of 6 mg/kg cisplatin ($n = 5$)

	DNA	CP	LMW	HMW
Amount (ng × 10^2):				
Liver	9.55 ± 1.4	38.01 ± 16.2	38.3 ± 8.1	60.1 ± 19.1
Kidney	2.84 ± 0.6	22.04 ± 18.3	37.7 ± 10	80.7 ± 34.7
Tumor	1.11 ± 0.7	18.71 ± 17.2	14.6 ± 4.2	34.5 ± 16.6
%ID/organ (× 10^{-2}):				
Liver	6.18 ± 1.1	24.57 ± 10.5	24.87 ± 6	38.8 ± 12.4
Kidney	1.6 ± 0.33	14.43 ± 12.5	24.12 ± 5.5	51.7 ± 24.1
Tumor	0.72 ± 0.45	12.32 ± 11.5	9.48 ± 3	22.5 ± 11.3
% ID/g (× 10^{-2}):				
Liver	0.57 ± 0.08	2.39 ± 1.1	2.28 ± 0.4	3.69 ± 1.2
Kidney	0.89 ± 0.23	8.2 ± 7.6	13.5 ± 3.4	29.2 ± 13.3
Tumor	0.18 ± 0.07	2.46 ± 1.4	2.72 ± 0.9	5.85 ± 1.3

LMW, low-molecular-weight cytosolic fraction; HMW, high-molecular-weight cytosolic fraction; CP, chromosomal protein fraction; %ID, fractional percentage of the total injected drug
Tissue weights in grams: liver, 11.01 ± 1.27 ($n = 5$); kidney, 1.81 ± 0.11 ($n = 5$); tumor, 4.12 ± 2.4 ($n = 5$)

access to standard laboratory diet and water. Tumor (Walker 256 adenocarcinoma) fragments (approximately 200 mg) were implanted subcutaneously into one flank of each rat in the tumor group. Rats that had palpable tumors after 7–10 days were selected for the study.

Experimental design. Prior to the administration of an i. v. bolus of cisplatin, the rats were anesthetized with a 1 : 1 ketamine/rompun mixture (20 mg/ml rompun, 100 mg/ml ketamine; 0.2 ml/250 g rat). Polyethylene cannulae were implanted into the jugular vein of each rat, which then received 6 mg/kg cisplatin in isotonic (0.9%) saline through the jugular vein. The rats were killed 4 h after administration of the drug. The tissues of interest (liver, kidneys, and tumor) were isolated, washed in isotonic saline, dried, weighed, and frozen. The DNA, chromosomal proteins (CP), and cytosolic fractions were isolated according to the method of Mamaur [17], with the following modifications: after the tissues had been ground and centrifuged, the supernatant phase was isolated; 1 ml 10% trichloroacetic acid was added to an aliquot of the supernatant (10 ml) and the suspension was centrifuged again. The acid-insoluble fraction (pellet) was termed the high-molecular-weight fraction (HMW), and the supernatant or acid-soluble fraction was termed the low-molecular-weight fraction (LMW). The pellet obtained after the first spin during the grinding procedure was lysed and resuspended in an organic phase [chloroform-isoamyl alcohol, 24 : 1. (v/v)] and centrifuged. The DNA was isolated from the aqueous supernatant phase, and the interface protein phase was termed the chromosomal protein (CP) fraction. The amount and purity of DNA in the preparation was determined spectrophotometrically at 260 nm. The DNA preparation was found to be mostly double-stranded, intact DNA, as determined by its hyperchromic shift after heat denaturation at 100° C.

For i. v. infusion, the animals were prepared in the same manner as described above. Cisplatin (6 mg/kg) was infused via the jugular vein at a rate of 1.5 mg/h per 250-g rat. At the end of 1 h, the infusion, was stopped and the cannula was washed with normal saline and sealed. At 4 h postinfusion, the animals were killed and the tissues were isolated as described above.

Chemical analysis. The DNA, CP, and cytosolic fractions were quantitatively analyzed for platinum by flameless atomization at 2,700° C in a heated graphite atomizer (Perkin Elmer 360/370 HGA 2200). Absorbance was measured at 265.9 nm. Aliquots (40/µl) of samples requiring no pretreatment (DNA and LMW) were analyzed by direct combustion in the furnace. Samples requiring pretreatment (CP and HMW) were prepared by digestion in 10 ml concentrated nitric acid and then heating to dryness. The dried residue was then taken up in a known volume of 0.1 *N* HCl (1–3 ml) and an aliquot (40/µl) was placed in the furnace for the quantitation of platinum in the sample. To correct for the matrix effect the DNA and samples were analyzed against a standard curve based on cisplatin-spiked DNA solutions and the CP and cytosolic fractions were analyzed against a standard curve based on spiked 0.1 *N* HCl solutions.

Results

After an i. v. bolus dose of 6 mg/kg cisplatin, 25%–30% of the total amount of cisplatin in the kidney and tumor tissue was localized in the nucleus and 70%–75% was localized in the cytosol (Table 1). The liver retained a higher amount in the nucleus than did the kidney or tumor tissues. Table 2 summarizes the subcellular localization of platinum in liver, kidney, and tumor tissues, expressed in absolute amounts (ng), %ID/organ, and %ID/g, respectively, where %ID is defined as the fractional percentage of the total amount of injected drug. Cisplatin localization in the kidney and liver was 5- to 6-fold that in the tumor. The binding of platinum to the chromosomal proteins of a given tissue was 5- to 10-fold that to the DNA of the same

Table 4. Localization of platinum in the subcellular fractions of liver and kidney tissues 4 h following the administration of an i. v. bolus dose of 6 mg/kg cisplatin in control (non-tumor-bearing) rats

	DNA	Chromosomal proteins
Amount (ng $\times 10^2$):		
Liver	4.16± 1.32	44.35± 13.3
Kidney	1.06± 0.1	10.44± 3.69
%ID/organ ($\times 10^{-2}$):		
Liver	2.38± 7.5	25.31± 7.37
Kidney	0.61± 0.06	5.96± 2.12
%ID/g ($\times 10^{-2}$):		
Liver	0.32± 0.1	3.38± 0.98
Kidney	0.38± 0.04	3.76± 1.34

DNA fractions, $n = 7$; chromosomal protein fractions, $n = 8$

tissue. The cytosolic fraction (LMW + HMW) in all tissues studied exhibited the highest localization of platinum, being highest in the kidney, followed by the tumor and the liver, respectively, as determined on a %ID/g basis.

Table 3 lists the subcellular localization of platinum in the liver, kidney, and tumor tissue following a 1-h infusion of cisplatin at a rate of 1.5 mg/h per 250-g rat (for a total dose of 6 mg/kg), expressed in absolute amounts (ng), %ID/organ, and %ID/g, respectively. The infusion data show the same pattern of platinum localization as seen after an i. v. bolus dose, i. e. uptake in the liver and kidney tissue was 5- to 6-fold that in the tumor tissue and uptake in the CP fraction was 5- to 10-fold that measured in the DNA of all tissues assayed. The maximal amount of platinum was localized in the cytosolic fraction (LMW + HMW). The %ID/organ localization of platinum in all tissues assayed was higher after i. v. infusion than after bolus injection. Based on %ID/g, the localization in all tumor and kidney fractions (DNA, CP, LMW, HMW) was higher after i. v. infusion than following an i. v. bolus.

Although a comparison of platinum deposition after i. v. bolus injection vs an infusion of cisplatin based on %ID/g appeared to reveal higher localization in all tissues and all fractions after i. v. infusion, a Student's *t*-test analysis using the BMDP statistical package showed no significant differences ($P >> 0.05$) between the two routes of administration. Inasmuch as the mean values show a trend of consistently higher values in all the fractions and tissues after i. v. infusion, it is possible that this trend could be statistically confirmed with a larger number of animals.

The subcellular distribution was also determined in control (non-tumor-bearing) rats to determine whether the tumor affected changes in the subcellular platinum distribution in surrounding tissues. Table 4 lists the absolute amount (ng), %ID/organ, and %ID/g of platinum found in the subcellular fractions after an i. v. bolus dose of 6 mg/kg cisplatin. A significantly higher uptake ($P < 0.05$) in the kidney DNA fraction and higher localization in the liver and kidney CP fractions were observed in tumor-bearing rats as compared with control values.

Discussion

There are several publications that suggest that the antitumor/cytotoxic effect of cisplatin is due to the inhibition of DNA synthesis by the formation of inter- and/or intrastrand cross-links or DNA-protein cross-links [6, 7, 10, 20, 23]. However, the effects of cisplatin interactions with chromosomal proteins and cytosolic ligands has only been marginally addressed. It is well known that the DNA in the eukaryotic cell nuclei occurs not in the free state but complexed to histone and nonhistone preoteins in an assembly consisting of strings of nucleosomal subunits [12]. Therefore, for cisplatin to bind the DNA, two conditions must be met: free cisplatin must reach the nucleus and accumulate there in adequate concentrations, and the DNA should be accessible to the free cisplatin for interaction to occur. Although it has been confirmed that Pt can bind to histone [14, 27] and nonhistone proteins [1], the possible role of proteins in the binding of cisplatin has not been elucidated. It is unclear whether the chromosomal proteins are a target for cisplatin or whether they just serve to protect the DNA by maintaining the tightly wound configuration normally necessary for its protection. Cisplatin primarily occurs as the aquated species within the cell, is acidic (pKa 6.3), [5] and thus could bind with basic proteins (histones). Foka and Paoletti [8] demonstrated in isolated systems that the binding rate constant of cisplatin to histones is double that of DNA and chromatin and that DNA and chromatin have similar rate constants.

Although the isolated CP fraction probably includes the platinum cross-linked to the protein-DNA fraction, the contribution of this component to the CP fraction is not expected to be extensive, inasmuch as the DNA yields from each tissue were 60%–80% of those expected and platinum localized in the CP vs DNA at ratios ranging from 4:1 to 12:1 (Table 1). Thus, most of the platinum in the CP is probably due to Pt-protein binding not involving DNA. This extensive localization of platinum in the CP (histones, nonhistones, and protamines) could potentially have two effects. First, the chromosomal proteins could bind the free cisplatin inside the nucleus, thereby protecting the DNA. Second, the irreversible platination of CP would cause a break in the CP assembly, thereby exposing the DNA to platination and eventually leading to the inhibition of DNA synthesis. If this were to happen, it would result in a linear relationship between CP uptake and DNA uptake, i.e., higher binding to the CP fraction should accompany a higher binding to DNA.

Extensive binding of Pt in the cytosol (up to 70%) was observed in the liver, kidneys, and tumor tissues. Others investigators [13, 25] have shown similar localization in kidney and liver tissues. The concentration of free cisplatin drops rapidly to form the mono-aquo complex at chloride ion concentrations of <0.04 mol/l [5]. Because the intracellular chloride ion concentration is lower (4 m*M*) than that in the blood circulation (103 m*M*) [28], cisplatin would be expected to exist primarily as the aquated specie(s) inside the cell. Inasmuch as the aquated species react more rapidly with nucleophilic groups in the mito-

chondria, microsomes, and lysosomes [1], this would suggest that the interactions in the cytosol can decrease the extent of DNA and chromatin platination. The data from the present study (Tables 2–3) suggest that the cytosolic interactions of Pt may be linked to the cytotoxicity of cisplatin, since only the kidney and tumor tissues show higher cytosolic localization of Pt and these are the tissues that are most affected by cisplatin.

The data (Tables 2, 3) calculated on a %ID/g basis illustrates that the kidney has the highest amounts of platinum in its DNA fraction, followed by the liver and tumor tissues. Others authors [21, 24] have shown the same order of magnitude for DNA platination, i.e., kidney, liver, then tumor, in rat models. In Table 2, the data based on %ID/g illustrates that cisplatin does not specifically bind the tumor DNA and CP, as the kidney and liver show higher uptake in these fractions than does the tumor. The localization of cisplatin in the cytosolic fraction (LMW + HMW) was determined to be highest in the kidney, followed by the tumor tissue, and was lowest in the liver tissue. Cisplatin has been reported to be cytotoxic to kidney and tumor tissue, with little toxicity toward the liver. The data (Table 2) suggests the possibility of a direct relationship between the level of cytosolic localization and the cytotoxicity of cisplatin.

Kulamowicz and Walter [13] have shown that the presence of proteins can decrease the platination of DNA. Thus, the platination of CP and DNA can only occur after a threshold concentration of cisplatin (or the aquo species) has been reached. Litterst [15] reported that when cytosolic protein was precipitated with acid, most acid-soluble platinum was bound to glutathione or other low-molecular-weight compounds. Metallothioneins and sulfhydryl-rich metal-binding protein also showed high binding of platinum. Choie et al. [4] also reported high concentrations of platinum in the microsomal fractions of liver and kidney of non-tumor-bearing rats. Sharma and Edwards [25] studied the binding of Pt to the metallothionein (MT)-like protein present in the cytosol. MT serves to immobilize metal ions, thus providing a protective barrier against heavy metal ions. The stimulation of MT biosynthesis as a response to metals such as cadmium [19], mercury [3], and gold [26] could also hold true for Pt, mearing that the concentrations of Pt in the cytosol and nucleus could be modulated.

Although DNA cross-linking is reported to be the principal mechanism for the cytotoxicity of cisplatin, it may not be its only mechanism of action. Macquet et al. [16] showed that the antineoplastic effects of cisplatin on L1210 cells can occur in the absence of DNA synthesis. These authors interpreted their results to indicate that active platinum coordination complexes may disrupt the partition of chromosomes during mitosis. Perturbation of the nuclear matrix and its association with replicating DNA can cause a functional modification of DNA interactions. This was supported by Banjar et al. [2], who reported that cisplatin cross-links nuclear protein to DNA in a cell-cycle-specific fashion.

The reason for higher localization of platinum in the nuclear fractions of tumor-bearing animals as compared with controls is unclear. A possible explanation is that because of the aggressive nature of the tumor, there is a breakdown in the capillary network in and around the region of the tumor, causing blood to pool in that region and thereby enabling more time for free cisplatin to be incorporated intracellularly.

The short elimination half-life of free cisplatin from blood (32 min [18]) and the plasma protein-binding half-life of free cisplatin (30 min [15]) should theoretically enable and i.v. infusion to deliver a higher amount of drug to the target (tumor) and other sites over an extended period of time as compared with an i.v. bolus injection. The results show a trend toward higher platination in all fractions of all tissues investigated after i.v. infusion vs i.v. bolus.

Jacobs et al. [11] indicated that the infusion of cisplatin over a period of 24 h reduced renal toxicity due to the slower rate of delivery of free cisplatin to the kidney. In contrast, the present study shows that the amount of cisplatin accumulated in the various subcellular fractions (kidney, liver, tumor) is higher after an i.v. infusion than after a bolus dose. The localization of cisplatin in the subcellular fractions reported in this study was at equilibrium (i.e., 4 h postadministration of cisplatin, when virtually no free cisplatin remains in the blood), and the data provide no information regarding the rate of cisplatin uptake by the various subcellular fractions. Further experiments are required to determine subcellular localization of cisplatin at different time points before equilibrium to explain the significance of the rate and amount of cisplatin delivery and the cytotoxicity of this drug.

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