Cancer Chemother Pharmacol (1991) 28: 159–165
034457049100078P

Cancer Chemotherapy and Pharmacology

Pentetration of carboplatin and cisplatin into rat peritoneal tumor nodules after intraperitoneal chemotherapy*

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Received 4 December 1989/Accepted 25 March 1991

Summary. Platinum distribution was studied in rat peritoneal tumors after i.p. treatment with equimolar doses of carboplatin and cisplatin. Low platinum concentrations (4 ppm) were detected in the periphery of the tumor after carboplatin treatment, whereas no platinum was detected 0.5 mm in from the periphery. In contrast, after cisplatin treatment, high platinum concentrations (29 ppm) were measured in the periphery of the tumor and moderate concentrations (14 ppm) were measured in the center. Only following increased carboplatin doses were low platinum concentrations detectable in the tumor. The total platinum concentration in the tumors was determined after equimolar administration of both drugs. In all, 7 times more platinum was detected after cisplatin treatment than after carboplatin treatment, and 10 times more carboplatin than cisplatin had to be injected to obtain comparable platinum concentrations in the tumors. When single cells were incubated with equimolar concentrations of carboplatin and cisplatin, 6–7 times more platinum was found in cells treated with cisplatin. However, pharmacokinetic studies favored i.p. administration of carboplatin because the clearance of this compound from the peritoneal cavity, expressed as $t_1/2\beta$, was lower than that of cisplatin (239 vs 78 min), resulting in an AUC in the peritoneal cavity for both total and ultrafiltered drug that was almost 3 times higher for carboplatin than cisplatin. The AUC for ultrafiltered carboplatin in plasma was 2-fold that for cisplatin ($2{,}801 \pm 210$ vs $1{,}334 \pm 431$ μM m). The present study demonstrated that in spite of the pharmacological advantages of carboplatin, its capacity to penetrate into peritoneal tumors and tumor cells is far lower than that of cisplatin.

* This work was supported by grant NKI 86-5 from the Dutch Cancer Society

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Introduction

Carboplatin (CBDCA), an analogue of cisplatin (CDDP), has shown appreciable activity against ovarian cancer [4, 39]. It is less nephrotoxic, neurotoxic and emetogenic than CDDP [4, 24, 33]. Because of its favorable toxicity profile, CBDCA has been subjected to preclinical studies [2, 17, 24] and clinical studies [1, 3, 7, 35, 37] aimed at exploring its antitumor activity as compared with that of CDDP.

A clear advantage of i.p. chemotherapy over i.v. therapy has been demonstrated in cancers restricted to the peritoneal cavity. Complete remissions were achieved after i.p. administration of CDDP in 30% of patients who suffered from ovarian cancer; all had previously been treated with high-dose i.v. CDDP [31, 36]. The toxicity of i.p. CDDP was acceptable at conventional doses (60–120 mg/m^2), and higher doses of up to 200 mg/m^2 could be given with concomitant i.v. administration of sodium thiosulphate [18, 36].

The successful use of CDDP in i.p. chemotherapy led to trials of i.p. CBDCA [6, 15, 29, 30]. The initial results of these studies indicated pharmacological advantages for CBDCA over CDDP, such as slower drug elimination from the peritoneal cavity and lower protein binding. This means that in terms of unchanged drug, peritoneal tumors are exposed to more CBDCA than CDDP via both the peritoneal cavity and the circulation. Considering its favorable toxicity profile, CBDCA is a promising candidate for i.p. chemotherapy.

We have previously demonstrated that a high drug concentration in the peritoneal cavity contributes to a large extent to the penetration fo CDDP from the peritoneal cavity directly into the tumor [26], which leads to better intratumoral drug distribution and finally results in better tumor responses. The purpose of the present study was to determine the penetration properties of CBDCA in tumor cells in vitro and in cancers restricted to the peritoneal cavity after i.p. treatment. Parallel studies using CDDP and CBDCA were performed to determine the intracellular Pt concentrations, the Pt tumor concentrations, the Pt distri-

bution within tumors and the pharmacokinetics after equimolar and higher CBDCA doses.

Materials and methods

Rats. Male WAG/Rij rats aged 8–12 weeks at the time of the experiments were obtained from the animal department of the Netherlands Cancer Institute. The animals were kept in a temperature-controlled room on a schedule of 12 h light/12 h darkness and were fed standard rat chow and tap water ad libitum.

Tumor. The CC531 tumor is a well-characterized carcinoma that originated in the colon of rats exposed to methylazoxymethanol [40]. In vivo the tumor grows both subcutaneously and intraperitoneally, forming small tumor nodules on peritoneal surfaces and in a final-phase ascites. In vitro the tumor cells are replated at a density of 1×10^5 cells in fresh Dulbecco's modified essential medium (DMEM) supplemented with 10% foetal calf serum (FCS, Flow Laboratories). The doubling time of the CC531 tumor cells in vitro is 16 h. Single-cell suspensions were prepared by enzymatic detachment of the CC531 cells using trypsin. Trypsin was inactivated by the addition of DMEM $\times$10% FCS. The solution was centrifuged and cell concentrations were prepared in phosphate-buffered saline (PBS). The viability of the cells was assessed by trypan blue exclusion.

Drugs. CDDP and CBDCA were supplied by Bristol Myers as Platinol and JM8, respectively.

Rat model. WAG/Rij rats were inoculated i.p. with 2×10^6 CC531 tumor cells in 2 ml PBS on day 0. At 4 weeks after inoculation, small tumor nodules were present in 80%–100% of the rats. Tumor nodules were situated on the diaphragm and peritoneum as well as on the mesothelium between the intestines; distant metastases were rare [27]. Treatment with CDDP or CBDCA was started at 28 days after inoculation. Tumors and various target tissues were collected at set times to determine Pt concentrations in tissues. Before tissue sampling, animals were killed with ether.

Flameless atomic absorption spectroscopy. A model AA40 atomic absorption spectrometer equipped with a GTA 96 graphite tube atomizer (with Zeeman background correction) from Varian (Victoria, Australia) was used for Pt analysis. Platinum concentrations were determined in plasma, peritoneal fluid, tumor tissue, tumor cells and target tissues. Samples were diluted (1:4, v/v, for plasma and ultrafiltrate) with a solution containing 0.2 M HCl and 0.15 M NaCl. Aliquots of tissue (about 200 mg) or $20-40 \times 10^6$ tumor cells were digested with 2.5 ml 65% HNO_3 at 170° C in a Parr Teflon-lined acid digestion (PTAD) bomb for 2 h. After cooling, the liquid was evaporated under a stream of air following the addition of 5 mg NaCl. The residue was dissolved in 0.2 ml 0.2 M HCl and 0.15 M NaCl. When necessary, the tubes were placed in an ultrasonic bath for 10 min. All standards were treated in the same way as samples that were diluted with or digested in the appropriate matrix. A four-stage heating program was used that consisted of drying at 110° C for 65 s, ashing at 1,400° C for 75 s, atomizing at 2,650° C for 3 s using maximal power, and conditioning at 2,550° C for 5 s. The inert gas was nitrogen.

CDDP and CBDCA uptake in tumor cells. Cells ($20-40 \times 10^6$) were incubated with equimolar concentrations of CDDP (4 μg/ml) and CBDCA (4.92 μg/ml) for 1 and 4 h at 37° C and were than harvested, washed twice with PBS and prepared for Pt determination.

Proton-induced X-ray emission. The proton-induced X-ray emission (PIXE) facility at the Eindhoven University of Technology was used to measure Pt concentrations at different depths within tumors. The technical conditions have been described elsewhere [12, 13, 20]. For the measurement of spatial distributions, cryostat sections of 40 μm were cut. After drying, the mass thickness was about 0.5 mg/cm^2. The freeze-dried sections were covered with aluminum foil and packed between polystyrene layers [11]. Calibration samples were prepared as follows: the polystyrene was loaded with a well-defined solution of cobalt acetylacetonate for the quantitative determination of the ratio of a well-known cobalt peak area and the maximal height of the bremsstrahlung, which appears at lower energy levels in the spectra. Furthermore, PIXE spectra were obtained from tumor sections taken from untreated rats. In these spectra, the germanium $K\alpha$ fluorescent peak area appeared to be equivalent to a concentration of 1.5 ppm; this backbround level was subtracted from the other values. Pt concentrations were determined in a line from the periphery to the center of the tumor in areas of 1,600 μm^2 (beam size, about 40 μm in diameter), with a distance of about 500 μm remaining between each point measured.

Comparison of Pt concentrations after i.p. chemotherapy with CDDP and CBDCA. WAG/Rij rats were inoculated with 2×10^6 CC531 tumor cells and were treated i.p. with CDDP (5 mg/kg) or CBDCA (6.15, 30 and 49.2 mg/kg) at 4 weeks thereafter. The drugs were injected i.p. in 20 ml 0.9% NaCl solution. Tumor tissue was collected at 4, 24, 48 and 168 h after treatment. Normal tissues from the liver, kidney, spleen intestines and lung were collected at 24 and 168 h after treatment for evaluation of the biodistribution of both drugs. Pt concentrations were determined by flameless atomic absorption spectroscopy (FAAS). The maximal tolerated dose (MTD) as determined by survival was set at 5 mg/kg for CDDP and 30 mg/kg for CBDCA. The low MTD of CDDP implied that in some studies in which a comparison was made between CDDP and CBDCA following equimolar treatment, a multiple dose schedule was required. The distribution of platinum in tumors was studied after treatment with CDDP (2×4 mg/kg CDDP) or CBDCA (2×4.9, 24.6 mg/kg). Tumor tissue was collected at 24 h after the last drug injection. The distribution of Pt was quantitatively determined by PIXE.

Pharmacokinetic studies. Pharmacokinetic studies were performed in WAG/Rij rats after cannulation of the carotid artery. CDDP (5 mg/kg) and CBDCA (6.15 mg/kg) were injected i.p. in 20 ml 0.9% NaCl. At different times after drug treatment, blood samples (300 μl) were taken from the carotid artery. Following plasma separation, Pt concentrations in plasma and in plasma ultrafiltrate obtained by filtration on an Amicon filter (cutoff, 10 kDa) were determined by FAAS. Clearance of drugs from the peritoneal cavity was studied in specimens of the instilled peritoneal fluid (150 μl) that were obtained at the same time as the blood samples. Corrections for depletion in volume were carried out in blood and peritoneal fluid by addition of the same volume (300 or 150 μl) of a 0.9% NaCl solution. No correction had been made for the reduction in the volume of peritoneal fluid. Areas under the plasma concentration-times curves (AUCs) were calculated by means of the trapezoidal rule in a two-compartment model [16]. Other pharmacokinetic parameters were calculated from the semilogarithmic plasma and peritoneal fluid concentration-time curves according to standard methods [16].

Statistics. Student's *t*-test was used to determine statistical significance; a value of $P > 0.05$ was considered to be non-significant.

Results

Platinum concentration in CC531 tumor cells and peritoneal tumors after CBDCA and CDDP treatment

Incubation of CC531 cells in vitro with equimolar concentrations of CDDP (4 μg/ml) and CBDCA (4.92 μg/ml) resulted in 6–7 times higher Pt concentrations in the CDDP-treated cells (Table 1). Pt concentrations in CC531 cells were comparable for both drugs following incubation with 39.8 μg/ml CBDCA and 4 μg/ml CDDP for 1 h or even 4 h (Table 1). Therefore, a CBDCA dose 10-fold that of CDDP is required to achieve comparable intracellular Pt concentrations (Table 1).

In tumor-bearing rats, concentrations of Pt in pritoneal tumors after treatment with CDDP (5 mg/kg) were 7 times

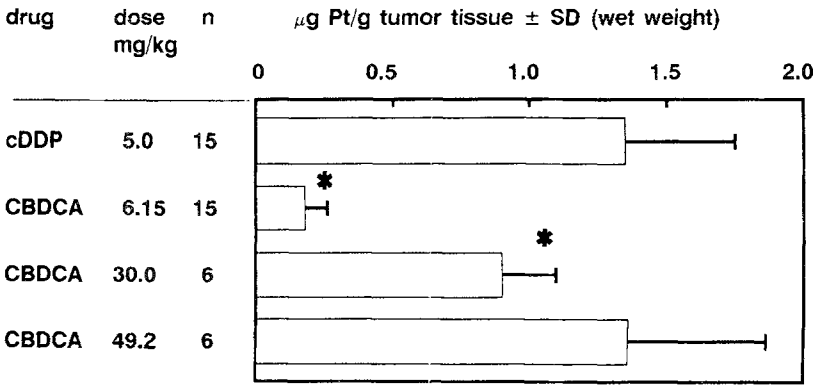

Fig. 1. Platinum concentrations (μg/g tumor) in peritoneal tumors following i.p. administration of 5 mg/kg CDDP and 6.15, 30 and 49.2 mg/kg CBDCA at 24 h after treatment. *n*, Number of rats; *, significantly different as compared with the tumor Pt concentration obtained after treatment with 5 mg/kg CDDP ($P < 0.05$)

Table 1. Pt concentration in CC531 cells after a 1- and a 4-h incubation with CDDP and CBDCA

Incubation time	Pt concentration (ng Pt/10^6 cells)		
	CDDP	CBDCA[b]	CBDCA[c]
1 h	2.73 ± 1.6	0.37 ± 0.2	2.4 ± 1.6
4 h	3.78 ± 1.8	0.56 ± 0.1	5.3 ± 2.1

Data represent the mean ± SD of 3 experiments
[a] 4 μg CDDP/ml
[b] 4.92 μg CBDCA/ml
[c] 39.8 μg CBDCA/ml

Table 2. Pt distribution in peritoneal tumors after i.p. administration of CBDCA and CDDP

Distance inward from the periphery (mm)	PT concentration (μg/g tumor tissue)		
	CBDCA (mg/kg)		cDDP 2 × 4 mg/kg
	2 × 4.9	24.6	
0.1	4 ± 2	8 ± 2	29 ± 4
0.5	3 ± 2	–	26 ± 4
1	ND	7 ± 1	25 ± 3
2	–	7 ± 1	–
2.5	ND	–	14 ± 2
3	–	7 ± 2	–

Number of rats = 3. ND, Not detectable

higher than those achived using an equimolar dose of CBDCA (6.15 mg/kg). At equitoxic levels (doses of 5 and 30 mg/kg for CDDP and CBDCA, respectively), higher Pt concentrations were also observed after CDDP treatment. Comparable Pt concentrations in peritoneal tumors were found after the CBDCA dose had been increased to 49.2 mg/kg (Fig. 1). Analogous to the in vitro situation, about 10 times more CBDCA than CDDP was also required in vivo to achieve the same intratumoral Pt concentration.

Distribution of platinum in peritoneal tumors after i.p. treatment with CBDCA and CDDP

Platinum levels were quantitatively determined in 40-μm tissue sections using PIXE in a line scan from the periphery of the tumor to the center. As shown in Table 2, intratumoral Pt concentrations were measurable after CBDCA treatment only following doses of >2 × 4.9 mg/kg CBDCA. Lower doses resulted in non-detectable Pt concentrations (data not shown).

A comparison of the intratrumoral Pt distribution after equimolar CBDCA and CDDP treatment revealed an impressive difference in tumor Pt concentrations. After treatment with CDDP (2 × 4 mg/kg) and CBDCA (2 × 4.9 mg/kg), the CDDP-treated tumor exhibited higher concentrations at the periphery as well as in the center of the tumor (Table 2). The difference in Pt concentration at the periphery represented about a factor of 7. The Pt concentration in the center of the CBDCA-treated tumor was too low to detect, which means that it was <2 μg/g tissue. Following a 2.5-fold increase in the CBDCA dose to 24.6 mg/kg, Pt could be detected in the center of the tumor, but at levels lower than those obtained using a low dose of CDDP (2 × 4 mg/kg).

Pt distribution patterns were different for the two drugs in these peritoneal tumors. For CDDP-treated rats, a concentration gradient was established from the periphery to the center of the tumor. However, no clear Pt concentration gradient was seen after i.p. treatment with CBDCA. Following treatment with 2 × 4.9 and 24.6 mg/kg CBDCA, Pt distribution was fairly homogeneous (Table 2).

An explanation for the difference in penetration capacity between these drugs might involve the difference in their solubility. We therefore determined the solubility of CBDCA and CDDP in chloroform ($CHCl_3$) and water. The coefficient $CHCl_3/H_2O$ for CDDP was <0.008 (solubility: in H_2O, 8.9 mM; in $CHCl_3$<0.071 mM) and that for CBDCA was <0.00004 (solubility: in H_2O, 50 mM; in $CHCl_3$, <0.002 mM). This indicates that CDDP is more soluble than CBDCA in lipids, which might imply better passage through membranes.

Biodistribution of CDDP

Tissues from the lung, liver, kidney, spleen and small intestines of rats were sampled after the administration of different doses of CBDCA (Table 3). The highest Pt concentrations were measured in the kidney, whereas levels observed in the lung, spleen, intestines and liver were low. Significantly higher Pt concentrations were found in all tissues measured, especially the kidney, following equimolar treatment with CDDP. Pt tissue concentrations mea-

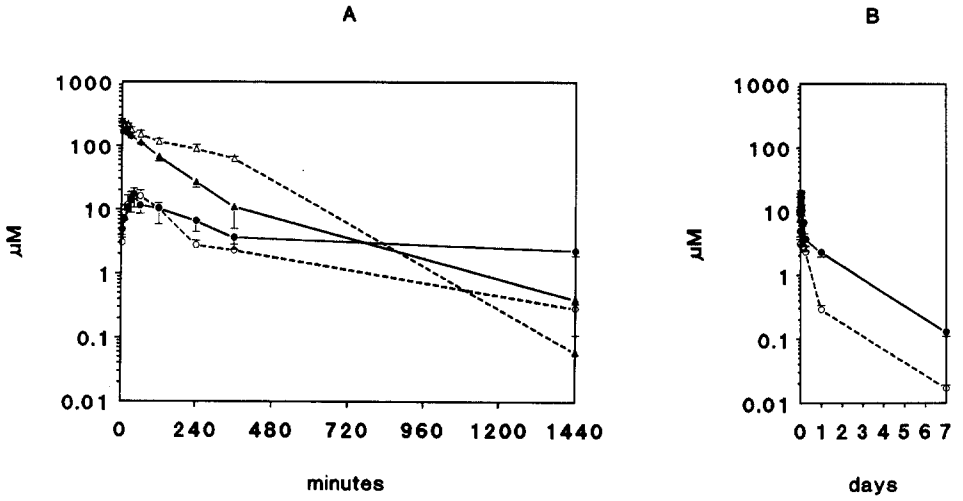

Fig. 2A. Semilogarithmic concentration vs time plots (0–24 h) of total Pt concentrations in the plasma and peritoneal fluid of rats after i.p. administration of 5 mg/kg CDDP (●, in plasma; ▲, in peritoneal fluid) and 6.15 mg/kg CBDCA (○, in plasma △, in peritoneal fluid). **Fig. 2B.** Semilogarithmic concentration vs time plots (1–7 days) of total CDDP (●) and CBDCA (○) in plasma after i.p. administration of 5 mg/kg CDDP and 6.15 mg/kg CBDCA to rats

Table 3. Biodistribution of CBDCA and CDDP

Drug (mg/kg)	Schedule (time)[a]	Platinum concentration in tissue (μg/g tissue)				
		Liver	Kidney	Spleen	Intestines	Lung
CBDCA (6.15)	24 h	0.56±0.4	2.1±0.4	0.4±0.1	0.2±0.1	0.3±0.2
CBDCA (6.15)	168 h	0.1 ±0.1	1.8±1.3	0.5±0.1	0.1±0.1	0.1±0.1
CBDCA (2×4.9)[b]	158 h	0.3 ±0.2	2.8±2.2	0.9±0.5	0.1±0.1	0.2±0.1
CBDCA (24.6)	24 h	2.6 ±1.5	10.5±3.1	3.3±1.3	0.8±0.3	2.4±1.5
CDDP (5)	24 h	3.3 ±0.9	13.5±2.7	2.6±0.4	1.3±0.2	1.8±0.2

[a] Time of tissue collection after last treatment
[b] Time between treatments, 168 h

Number of rats = ⩾6

Table 4. Pharmacokinetic data in the plasma and pertioneal cavity of rats after i.p. treatment with equimolar doses of 6.15 mg/kg CBDCA and 5 mg/kg CDDP

Parameter	CBDCA		CDDP	
	Total Pt	Free Pt	Total Pt	Free Pt
AUC_{plasma} (0–1,440 min)	4.1×10^3 $\pm1.6\times10^3$	2.8×10^3 $\pm0.2\times10^3$	5.8×10^3 $\pm0.8\times10^3$	1.3×10^3 $\pm0.4\times10^3$
t_{max} (min)	60±17	60±8.6	40±8.6	40±10
c_{max} (μM)	16±5.6	17.3±1.2	17±2.6	11±1.7
$t^1/_2\beta_{plasma}$ (60–360 min)	99±20	70±14	160±32	74.7±15
$AUC_{p.c.}$ (0–1,440 min)	75×10^3 $\pm5\times10^3$	57×10^3 $\pm12\times10^3$	28×10^3 $\pm7\times10^3$	20×10^3 $\pm2\times10^3$
$t^1/_2\beta_{p.c.}$ 30–360 min)	239±35	195±52	78±23	65±13

p.c., Peritoneal cavity; AUC, area under the concentration × time curve (μM m). Results were obtained from at least four concentration × time curves for each drug

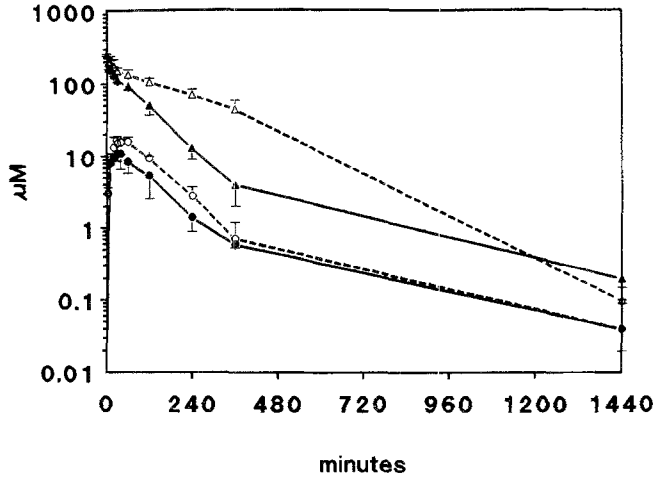

Fig. 3. Semilogarithmic concentration vs time plots (0–24 h) of ultrafiltered Pt in the plasma and peritoneal fluid of rats after i. p. administration of 5 mg/kg CDDP (●, in plasma; ▲, in peritoneal fluid) and 6.15 mg/kg CBDCA (○, in plasma. △, in peritoneal fluid)

sured after a CBDCA dose of 24.6 mg/kg were similar to those achieved after treatment with 5 mg/kg CDDP.

Pharmacokinetics of CBDCA and CDDP in plasma and peritoneal fluid after i. p. administration

The concentration-time curves for CBDCA and CDDP in plasma and peritoneal fluid differed as shown in Fig. 2 and 3. AUCs for both total and ultrafiltered Pt were higher in peritoneal fluid after CBDCA treatment than those obtained after CDDP administration (Table 4, Figs. 2, 3). This can be explained by the finding that CBDCA was eliminated from the peritoneal cavity more slowly than CDDP, with a $t^1/_2\beta$ value of 239 min being found for CBDCA and that of 78 min being recorded for CDDP (Table 4). In Fig. 2, the total Pt concentrations in plasma are also shown. The c_{max} for CBDCA occurred about 20 min later than that for CDDP. CBDCA also left the circulation faster than CDDP ($t^1/_2\beta$ 99 and 160 min, respectively). Differences in the levels of free (ultrafiltered) drug between CBDCA and CDDP were comparable with those in total drug. Figure 3 and Table 4 demonstrate that CBDCA appeared later in plasma than did CDDP, that the maximal ultrafiltered Pt concentration (c_{max}) achieved in plasma was significantly higher after CBDCA treatment than after CDDP administration and that the AUC for ultrafiltered CBDCA was significantly higher than that for ultrafiltered CDDP. All of these results indicate pharmacological advantages for CBDCA in comparison with CDDP.

Table 5. Tumor-to-plasma ratios of platinum following i. p. administration of CBDCA and CDDP to rats

Drug	Pt concentration		Tumor : plasma ratio
	Plasma (ng/m)	Tumor (ng/g tumor)	
CBDCA 24 h[a]	57 ± 11	180 ± 83	3.1
CBDCA 168 h	3 ± 1	104 ± 33	34.6
CDDP 24 h	450 ± 70	1,760 ± 260	3.9
CDDP 168 h	25 ± 5	1,600 ± 200	64

[a] Interval between treatment and sample collection
Pt tumor and Pt plasma concentrations were measured in ≥6 animals

Pharmacokinetics of CBDCA and CDDP in peritoneal tumors after i. p. administration

Platinum from CBDCA or CDDP persits in the peritoneal tumor for at least 7 days. This, coupled with the relatively short half-life of CBDCA and CDDP in plasma, results in a progressive increase in the platinum tumor-to-plasma ratio with time. Table 5 indicates that the Pt ratios (Pt tumor concentration : Pt plasma concentration) following CBDCA and CDDP treatment were similar during the initial decay phase. Within 7 days, however, the tumor-to-plasma ratio of Pt concentration after CDDP treatment exhibited a 3.9-fold increase to 64, whereas it showed a 3.15-fold increase to 34.6 following CBDCA administration. This indicates that more Pt was retained in the tumors after CDDP treatment. In line with these data were the differences in the β-phase half-life of tumor Pt concentrations. The $t^1/_2\beta$ value measured in a two-compartment open model over the period from 24 to 168 h after treatment was almost 6 times higher for CDDP (1,018 h) than for CBDCA (171 h).

Discussion

The successful use of i. p. CDDP in cancers restricted to the peritoneal cavity and the use of CBDCA in i. v. chemotherapy led to trials of i. p. CBDCA [6, 15, 29, 30]. Since the penetration of CDDP from the peritoneal cavity into tumors is of major importance for the ultimate result [26], the uptake of CBDCA and its penetration into peritoneal tumors were examined extensively in the present study.

Both in cultured cells in vitro and in peritoneal tumors in vivo, 7 times more Pt could be detected after CDDP treatment than after equimolar treatment with CBDCA. The CBDCA dose had to be increased by a factor of 10 to give similar Pt concentrations in both cells and tumors. In our peritoneal tumor model in the rat, the MTD of CBDCA was only 6-fold that of CDDP. When rats were treated at these levels, tumor Pt concentrations measured after CBDCA treatment were lower than those found after CDDP administration. The difference in the uptake of CBDCA and CDDP by cells and peritoneal tumors can be explained by the difference in the chemical structure of the two drugs. First, CBDCA has a higher molecular weight (371.3 kDa) than does CDDP (300.6 kDa), which could hinder the diffusion of CBDCA into tumor tissue. However, Dedrick et al. [9] have indicated that the influence of the molecular weight of drugs on their tissue diffusion is of only limited importance. These authors calculated that a difference in molecular weight of almost a factor of 100, e. g. between urea and inulin, would negatively affect the ultimate result by only a factor of 1.5, because other processes such as peritoneal and tumoral clearance of the drug have a positive influence on the ultimate drug concentration.

A second difference in the chemical structure of these drugs in volves their lipid solubility [10]. The lipid/water

distribution coefficient for CBDCA is <0.00004 and that for CDDP is <0.008. This chemical property makes the passage of CBDCA through membranes more difficult than that of CDDP [15]. Diffusion into peritoneal tumors directly from the peritoneal cavity after i.p. administration is probably affected in the same way as passage through membranes. Membranes have to be crossed in both cases, even if CBDCA is transported into the peritoneal tumor via the interstitial space. Kerr and Kaye [22] also underlined the importance of drug solubility, demonstrating in a tumor spheroid model that the penetration capacity of cytostatic drugs is related to their lipid/adequeous solubility.

A third difference concerns the activation of both drugs, which is chemically mediated and requires aquation of both the CDDP and the CBDCA molecules [23]. This is probably the rate-limiting step in the platination of macromolecules [5]. Carboplatin is thought to be activated in the same way as cisplatin, although the substitution of the bidentate cyclobutane dicarboxylate ligand of CBDCA for the two chloride ligands of CDDP, renders the former compound 10–20 times less reactive [5, 23].

In the present study, these effects were demonstrated in the difference between CBDCA and CDDP in plasma clearance and in the resultant Pt tumor concentrations. These drugs are excreted predominantly via the kidney, and the difference in $t^1/_2\beta$ observed in plasma was probably due to the more rapid and extensive binding of the CDDP molecules to macromolecules, resulting in a reduction in the amount of drug available for urinary excretion. The same phenomenon probably occurs in tumors. Pharmacokinetic analyses of concentration-time data in tumor tissue and in plasma (Table 5) demonstrated a difference in the tumor-to-plasma ratio between CBDCA (ratio = 34.6) and CDDP (ratio = 64) after 7 days. The faster body clearance (Table 4) lower protein binding in the interstitial spaces of peritoneal tumors and slower penetration into tumor cells exhibited by CBDCA are probably responsible for its more rapid and better clearance from the tumor [22]. The percentage of the delivered dose of drug that binds to DNA remains unknown; however, for the formation of the same number of CBDCA-DNA and CDDP-DNA intrastrand adducts, about 20 times more CBDCA than CDDP is required in the nucleus [23]. Translated to our rat model, this would mean that about 100 times more CBDCA than CDDP would have to be injected to produce an equal number of Pt-DNA adducts.

The pharmacokinetic parameters for CBDCA and CDDP are in close agreement with those previously reported in humans [6, 15, 30, 36]. The AUCs for CBDCA in terms of both total and ultrafiltered Pt in the peritoneal cavity were higher than those for CDDP, the clearance of CBDCA from plasma was faster than that of CDDP and the AUC for ultrafiltered CBDCA in plasma was 2-fold that obtained for CDDP. This indicates that the tumor exposure to drug via both the peritoneal cavity and the blood circulation after CBDCA treatment was better than that obtained following CDDP administration, although this did not lead to higher tumor Pt concentrations. The results of our tissue-distribution studies are in agreement with previous findings of Siddik and co-workers [34].

It is known that the interstitial pressure in tumors is high in the center and lower in the periphery [19]. One would therefore expect interstitial fluid motion to be directed from the center of the tumor outward to the periphery. Cytostatic drug molecules at the tumor periphery have to overcome this outward convection before they can penetrate into the tumor by diffusion. Since the diffusion coefficient depends on the molecular weight of the drug used an increase in molecular weight might be expected to decrease the diffusion distance [19]. However, the relatively small difference in molecular weight between CBDCA and CDDP does not explain the difference in Pt concentration measured at the periphery of the tumor. It seems that in contrast to CDDP, CBDCA does not penetrate the tumor. Almost no Pt was detected in the periphery of peritoneal tumors after treatment with CBDCA. Equimolar CDDP treatment resulted in high Pt concentrations in the periphery. The drug retained in the tumor is the net result of diffusion and resultant binding to tissue macromolecules. If diffusion occurs and the binding is rapid, high Pt concentrations can be detected in the periphery of the tumor; however, if diffusion hardly occurs and binding to macromolecules goes slowly, as in the case of CBCDA, no concentration gradient is formed. In view of a previous study in which direct penetration of CDDP into peritoneal tumors was put forward as the advantage of i.p. therapy over i.v. treatment [25, 26], the present study suggests that CBDCA is not ideal for i.p. use.

CBDCA has demonstrated activity in second-line systemic chemotherapy for ovarian tumors in patients who have relapsed after an initial response as well as in some ovarian tumors that are resistant to cisplatin [21]. CBDCA is more hydrophilic than CDDP, which results in a slower membrane passage as demonstrated by the clearance rate of both drugs in the peritoneal cavity. Peak plasma concentrations could therefore be important in obtaining a high drug-concentration gradient in the interstitial tissue around blood vessels. Elferink et al. [14, 15] have studied the pharmacokinetics of CBDCA in humans after i.p. and i.v. administration and demonstrated that the peak concentrations in plasma following i.p. administration were approximately 4 times lower than those obtained after i.v. administration. All other pharmacokinetic parameters were comparable [15]. The lower peak plasma concentrations observed after i.p. administration of CBDCA might result in lower intratumoral drug concentrations, which are not compensated by direct penetration of CBDCA from the peritoneal cavity into peritoneal tumors. This can finally result in a lower response rate for i.p. CBDCA as compared with i.p. CDDP; initial clinical results indicate lower rates of complete remission for CBDCA as compared with i.p. CDDP [32, 38].

In conclusion, the aim of i.p. chemotherapy is to eradicate established micrometastases in the peritoneal cavity after initial treatment of the primary tumor. Given the previous finding that drug penetration into tumor tissue is of major importance to the achievement of the ultimate goal [8, 26, 28], it is apparent from the present study that CBDCA, which has poor penetration capacities, may not be as active as CDDP in i.p. chemotherapy.

Acknowledgements. We thank Dr. F. A. Stewart for stimulating discussions and for his critical reading of the manuscript.

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