Cancer Chemother Pharmacol (1986) 17: 133–136

Cancer Chemotherapy and Pharmacology

Pharmacokinetics and plasma protein binding of two platinum cytostatics CHIP and CBDCA in rats

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Summary. Plasma protein binding and pharmacokinetic parameters of CHIP (*cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine platinum IV) and CBDCA (*cis*-diammine-1,1-cyclobutane dicarboxylate platinum II) were investigated in male Wistar rats. The plasma clearance of total and non-protein-bound platinum was determined and compared with that of ^{99m}Tc-DTPA. For binding experiments, a novel, simple, and quick method based on adsorption of non-protein-bound platinum species to charcoal was used. The clearance of total platinum after CHIP and CBDCA administration was markedly lower than the glomerular filtration rate (determined as the clearance of ^{99m}Tc-DTPA). The renal clearance of non-protein-bound platinum corresponded to 168% and 50% of the glomerular filtration rate for CHIP and CBDCA, respectively. These studies suggested that CHIP was excreted by the rat kidney.

Introduction

cis-Diamminedichloroplatinum (cisplatin) is an important antineoplastic drug exhibiting activity against a wide variety of human malignancies [12, 13]. However, its clinical use is limited mainly by nephrotoxicity, which stimulated the development of second-generation platinum drugs free of this side effect for SC administration. CHIP and CBDCA have been found to be less markedly nephrotoxic alternatives to cisplatin. The pharmacokinetics of these drugs have been studied in animals and patients [6, 10, 11, 14, 15], but data on the role of plasma protein binding in the pharmacokinetics of CHIP and CBDCA are scanty. It was suggested by the cisplatin pharmacokinetics studies that non-protein-bound platinum is probably the biologically active platinum species [4, 5].

In the present study a simple method for determination of CHIP and CBDCA binding to plasma proteins was developed. Plasma concentrations of total and non-protein-bound platinum were assayed after CHIP and CBDCA administration to rats, and the plasma clearances of total and non-protein-bound platinum were compared with the glomerular filtration rates.

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Materials and methods

The animals used were male Wistar rats weighing 180–210 g, fed with standard pelleted food and water *ad libitum.*

CHIP and CBDCA were kindly provided by Lachema, Brno, Chechoslovakia.

In some in vitro experiments comparing the results of different methods of protein binding determination, radiolabeled (U-^{14}C) CHIP was also used. Radiolabeled CHIP (radiochemical purity greater than 97%) was kindly provided by the Institute for Research, Production and Application of Radioisotopes, Prague.

The complex of ^{99m}Tc with DTPA (diethylenetriamine-penta-acetic acid) was prepared by mixing 0.1 ml 20 mmol/l $SnCl_2$ in 0.12 mol/l HCl with 1 ml 40 mmol/l DTPA in saline (pH adjusted to 7 by 1 mol/l NaOH) and adding 2.8 ml pertechnetate eluate (activity 100–200 MBq/ml). The radiochemical purity of the complex was higher than 99%.

Rats received injections of 20 mg/kg CHIP or CBDCA (10 mg/ml in saline and 10% dextrose, respectively) into the femoral vein under light ether anesthesia. At predetermined time intervals blood was taken from the exposed carotid artery of anesthetized rats into heparinized glass test tubes, after which the plasma was immediately separated by centrifugation for 1 min and assayed for total and bound platinum.

The pharmacokinetics of ^{99m}Tc-DTPA was examined in a similar way after administration of 0.2 ml solution of ^{99m}Tc-DTPA intravenously per animal, which corresponds to 2 μmol DTPA and 0.1 μmol $SnCl_2$.

For the determination of protein-bound platinum 50 mg charcoal was added to 0.5 ml plasma and the mixture was vigorously shaken for 2 min; charcoal was then removed by centrifugation for 1 min and the solution assayed for platinum concentration.

Control data of plasma protein binding were obtained by the equilibrium dialysis method [9] performed for 3 h, by the trichloroacetic acid (TCA) precipitation procedure [3], and by ultrafiltration across a semipermeable membrane (Priesvit, Chemosvit, Svit) for 20 min at 2000 *g*.

The samples were assayed for platinum by flameless atomic absorption spectrophotometry (Perkin-Elmer, model 4000).

The activity of ^{99m}Tc and ^{14}C in plasma was measured on the beta-gamma spectrometer NE 8312 (Nuclear Enterprises Ltd, Edinburgh, UK).

The pharmacokinetic parameters were calculated by linear regression analysis. Experimental data could be fitted into the function.

$$C = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t)$$

or

$$C = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t) + C_3 \exp(-\lambda_3 t)$$

representing the linear two- and three-compartment open pharmacokinetic models respectively [16], where C_i is the zero time hybrid concentration constant and λ_i is the hybrid rate constant. The pharmacokinetic volume of the central compartment, V_1, the total-body clearance of drug from plasma, CL, and the half-lives of individual processes, $T_{½\,(\lambda i)}$, were calculated using the equations

$$V_1 = \frac{\text{Dose}}{\sum_{i=1}^{n} C_i}$$

$$Cl = \frac{\text{Dose}}{AUC}, \text{ where } AUC = \sum_{i=1}^{n} \frac{C_i}{\lambda_i}$$

$$t_{1/2(\lambda_i)} = \frac{\ln 2}{\lambda_i}$$

The renal clearance CL_R of free platinum was calculated using the equation

$$CL_R = \frac{\text{Platinum excreted in urine (0–24h)}}{\text{Free platinum area under the curve}}.$$

Results and discussion

Charcoal sorption is used to remove low-molecular-weight endogenous or exogenous substances from plasma [2] with no apparent sorption or modification of proteins. We found in preliminary experiments that more than 99% of CHIP or CBDCA is removed from solutions free of proteins by the treatment described for these experiments whereas practically no decrease of platinum concentration was observed in charcoal-treated rat plasma obtained 4 days after IV administration of CHIP or CBDCA.

Comparison of protein-binding data obtained by different methods of determination showed good agreement with respect to the results of charcoal treatment and ultrafiltration methods. Equilibrium dialysis data may be affected by the decrease in the free CHIP fraction in plasma during the period necessary to establish the dialysis equilibrium (3 h). The higher value of CHIP binding determined by the TCA precipitation method in the case of the low free CHIP fraction may be due to incomplete removal of free CHIP from the protein precipitate. It can be concluded from these results that no significant desorption of the bound platinum drug from plasma proteins occurs with charcoal treatment. The very low bound fractions of both drugs determined in the shortest tested time interval (Table 1) suggests that very rapid and reversible binding to plasma proteins, which is typical of most common drugs, does not play a significant role in the case of the platinum drugs tested.

The plasma concentrations of free and total platinum over time (Figs. 1 and 2) were evaluated using a two-compartment open model for the free fraction and a three-compartment open model for total platinum. The calculated parameters are shown in Tables 2 and 3. The glomerular filtration rate (GFR) was expressed in terms of the ^{99m}Tc-DTPA two-compartment pharmacokinetics (Table 4).

The Pt (IV) complex CHIP, when compared with CBDCA, showed a lower distribution volume of the central compartment, a faster initial decrease of the plasma level, and a higher elimination rate of free platinum (λ_2-phase), with a resulting higher plasma clearance of free platinum. This clearance, like the renal clearance of CHIP, is more than twice as high as the GFR, which indicates the tubular secretion of CHIP.

The parameters of the λ_1 and λ_2 phases are seen to be very close when the model parameters for free and total platinum are compared. This shows that the total platinum pharmacokinetics in the λ_1 and λ_2 phases is mostly characterized by the fate of free platinum.

The slowest, λ_3, phase is similar for both complexes and apparently reveals the elimination of bound platinum. The kinetics is probably controlled by the detachment of platinum and/or by the degradation of protein molecule with bound platinum.

The total plasma clearance of CHIP determined by the decrease in the plasma level of total platinum amounts to only 7% of the value obtained from the free platinum concentration. This is the result of the high area-under-the-curve value for total platinum, i.e., of the binding to plasma proteins. Even with low platinum concentrations the

Table 1. CHIP and CBDCA binding to rat plasma proteins after 20 mg/kg drug: Comparison of different methods of plasma protein-binding determinations (means ± SD)

Drug	Time after administration to rats	Percentage of free drug			
		Charcoal treatment	Ultrafiltration	TCA precipitation	Equilibrium dialyses
CHIP	0[a]	99.2 ± 0.1	100.0 ± 4.0	97.7 ± 1.6	92.6 ± 2.7
	20 min	61.8 ± 0.3	58.8 ± 2.1	65.6 ± 3.5	43.4 ± 3.9
	120 min	11.3 ± 3.1	14.6 ± 1.8	17.2 ± 2.8	7.9 ± 0.4
CBDCA	0+	97.1 ± 0.7	93.5 ± 9.5	–	–
	120 min	71.2 ± 5.0	66.7 ± 8.2	–	–

[a] Drug was added to rat plasma in vitro at the concentration of 10 mg/l and binding experiments were started after 1 min incubation

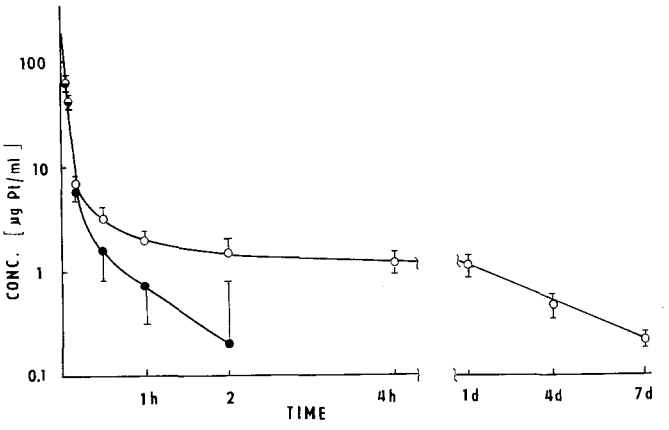

Fig. 1. Decay of Pt plasma level in rats given 20 mg/kg CHIP intravenously (O, total Pt; ●, free Pt)

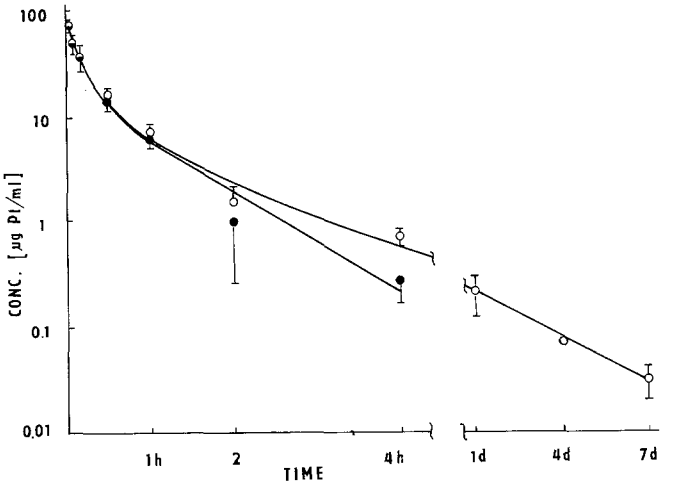

Fig. 2. Decay of Pt plasma level in rats given 20 mg/kg CBDCA intravenously (O, total Pt; ●, free Pt)

Table 2. Model-dependent parameters of CHIP and CBDCA pharmacokinetics in rats after IV administration of 20 mg/kg (9.36 mg Pt/kg for CHIP and 10.52 mg Pt/kg for CBDCA)

Parameter	CHIP		CBDCA	
	Total platinum	Free platinum	Total platinum	Free platinum
C_1 (μg Pt/ml)	178	168	63.7	58.4
C_2 (μg Pt/ml)	3.4	2.8	17.4	18.2
C_3 (μg Pt/ml)	1.36		0.290	
λ_1 (min^{-1})	0.400	0.376	0.107	0.108
λ_2 (min^{-1})	2.54×10^{-2}	2.22×10^{-2}	1.67×10^{-2}	1.86×10^{-2}
λ_3 (min^{-1})	1.80×10^{-4}		2.25×10^{-4}	
$t_{1/2(\lambda_1)}$ (min)	1.73	1.84	6.49	6.41
$t_{1/2(\lambda_2)}$ (min)	27.3	31.3	41.4	37.3
$t_{1/2(\lambda_3)}$ (hrs)	64.3		51.3	

Table 3. Model-independent parameters of CHIP and CBDCA pharmacokinetics in rats (dose as in Table 1)

Parameter	CHIP		CBDCA	
	Total platinum	Free platinum	Total platinum	Free platinum
V_1 (ml kg^{-1})	50.8	54.5	129	137
CL (ml min^{-1} kg^{-1})	1.14	16.2	3.60	6.92
CL_R (ml min^{-1} kg^{-1})		12.9		3.85
$f_{e(24\,h)}$[a]	0.862		0.623	

[a] Fraction of Pt excreted in urine in time interval 0–24 h

Table 4. Some pharmacokinetic parameters of ^{99m}Tc-DTPA in rats

Parameter	^{99m}Tc-DTPA
V_1 (ml kg^{-1})	115
CL (ml min^{-1} kg^{-1})	7.66
$t_{1/2(\lambda_1)}$ (min)	2.8
$t_{1/2(\lambda_2)}$ (min)	13.5

area is large, because of the slow release from the bound state. All these results support the conclusion that CHIP, similarly to cisplatin [1, 7, 8], is subject to active tubular secretion.

With CBDCA at longer time intervals, the platinum concentration and the area under the curve were smaller and the total platinum clearance exceeded 50% of the value obtained for the decrease of free platinum in plasma.

The above results show that the binding to plasma proteins should be considered in pharmacokinetic studies even with a low bound fraction. After both CHIP and CBDCA, free platinum dominates the fast elimination shortly after the IV administration, whereas at later time points (over 4 h) the bound platinum becomes the controlling fraction.

Acknowledgements. The authors wish to thank the firm Lachema, Brno, for the gifts of the drugs used and for the AAS measurement and Dr J. Filip, from the Institute for Research, Production and Application of Radioisotopes, Prague, for the kind donation of radiolabeled CHIP. The authors also wish to thank Mrs J. Hoderová for technical work and Dr B. Mánek for language correction.

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Received April 1, 1985/Accepted November 26, 1985