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SHORT COMMUNICATION

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Determination of platinum in plasma of patients affected by inoperable lung carcinoma treated with radiotherapy and concurrent low-dose continuous infusion of *cis*-dichlorodiammine platinum(II)

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Abstract Platinum microquantities were determined in plasma of patients affected by lung carcinoma during treatment with radiotherapy (RT) and concurrent low-dose continuous infusion of *cis*-dichlorodiammineplatinum(II) (CDDP). RT was given at 50 Gy in continuous course; CDDP was continuously infused at 4 mg/m^2 daily for 100 h/week for 5 weeks, and the infusions were separated by 68 h of rest. The percentage of free drug versus total drug in plasma was about 3%. It did not vary with therapy duration and was not significantly different from that found in 5-day continuous infusions at much higher daily doses. Nevertheless, maximal values of free Pt in plasma were very low and agreed with the low level of CDDP toxicity encountered on the present administration schedule.

Key words CDDP · Continuous venous infusion
Lung carcinoma

Introduction

High-dose radiation therapy (RT) is the treatment of choice for inoperable locoregional lung carcinoma but, unfortunately, survival probabilities are dismal. Only 5% or less of the patients who respond to treatment (about one-half) are long-term survivors [4, 8]. Innovative treatments are currently designed to increase the rate of response and, therefore, the correlated rate of cured patients.

The concurrent administration of *cis*-dichlorodiammineplatinum(II) (CDDP) together with RT enhances the radiation-induced cell kill. A three-arm randomized clinical trial was recently reported [10] in which all patients underwent RT (55 Gy delivered in a split course). Of these, one arm received concurrent weekly bolus administration of CDDP (30 mg/m^2), whereas another one received concurrent administration of CDDP in repeated daily doses (6 mg/m^2 daily, 5 days/week). In both cases, CDDP increased local control, but drug fractionation proved to be more effective. Survival in the RT/daily-CDDP group was 54% at 1 year, 26% at 2 years, and 16% at 3 years as compared with 46%, 13% and 2%, respectively, in the RT group. However, the price of the survival advantage as regards toxicity was defined as substantial by the authors [10].

We tested CDDP given in a 120-h/week repeated continuous intravenous infusion (RCVI) at a daily dose of 6 mg/m^2 repeated for 5 weeks, concurrently with 50 Gy RT given in a split course in one phase II study (Bedini, submitted for publication). Adjuvant surgery was performed in about one-half of the patients. We obtained 1-, 2-, and 3-year survival rates of 63%, 37%, and 24%, respectively. In all, 1 of the initial 38 patients was assessed with a pathological complete response. In a further phase II study [2] consisting of 50 Gy RT given in continuous course over 5 weeks and concurrent 100-h/week RCVI of CDDP given at a daily dose of 4 mg/m^2, we assessed a pathological complete response in 19 of 80 patients. In both of these experiences, toxicity was mild to moderate. These data coming from clinical studies suggest that radiochemotherapy schedules could be further optimized in terms of both effectiveness and toxicity.

An understanding of pharmacokinetic parameters appeared mandatory to rationalize the efficacy of this schedule and to optimize its therapeutic index. Analytical

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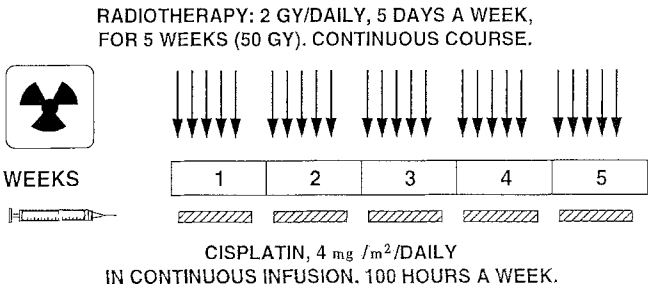

Fig. 1 Treatment schedule

problems due to the low levels of drug in the blood had to be preliminarily solved; separation, identification, and quantification methods for platinum had to be improved in terms of the precision of measurements. The present paper considers the experimental details of the analysis in plasma of Pt trace levels as separated and quantified in the two forms of "total" and "free" (unbound to proteins) element [1]. A preliminary pharmacokinetic analysis aimed at reproducing Pt levels from our RCVI schedule was also conducted.

Patients and methods

Selection of patients and administration procedure

A total of 22 patients affected by inoperable lung cancer were entered in the present study. They were subjected to therapy consisting of (a) RT given at 2 Gy daily for 5 days (from Monday to Friday), repeated for 5 weeks in sequence; and (b) chemotherapy consisting of RCVI with CDDP (4 mg/m^2 daily) for 100 h/week (from Monday to Friday), repeated 5 weeks in sequence. A detailed scheme is reported in Fig. 1.

Sampling procedure

Blood samples were drawn twice a week, just before the start of the continuous infusion on Monday and after the completion of the infusion on Friday. Two drops of heparin added to 6 ml of blood prevented blood coagulation well without causing any significant change in the Pt concentration in samples. Then, samples were centrifuged at 2,500 rpm for 15 min at 4 °C. Plasma (about 3 ml) was separated from the red cells and subdivided into two portions. Of these, portion A (about 1.5 ml) was centrifuged at 5,000 rpm for 90 min at 4 °C using Centriflo ultrafiltration membrane cones having a cutoff value of 50,000 Da (Amicon Corp., USA). Further centrifugation was performed at 5,000 rpm for 60 min at 4 °C on Centricon-10 micro-concentrators (Amicon Corp., USA) having a cutoff value of 10,000 Da. However, no residual fraction was obtained. The ultrafiltrate was stored at –20 °C until the analysis of free Pt. As regards portion B (at least 1 ml), this was stored at –20 °C until the analysis of total Pt.

Analytical procedure

Samples were thawed in a warm bath immediately before analysis. Total Pt determinations were accomplished by inductively coupled plasma-atomic emission spectrometry (ICP-AES) by diluting samples 1:3 (vol/vol) with Milli-Q (Millipore) water. No detectable level of the element was measured in water used for dilution. Calibration was performed using Pt(II) standard solutions in Milli-Q water. Matrix effects due to plasmatic components proved to be irrelevant within the concentration range under investigation. The advantages of ICP-AES as for the dynamic concentration range (linear responses were observed for 4 orders of magnitude) and the absence of matrix effects have been described elsewhere [5]. Basic information on the apparatus and experimental conditions is given in Table 1.

Free Pt determination was carried out by inductively coupled plasma-mass spectrometry (ICP-MS) after 1:4 (vol/vol) dilution of samples with Milli-Q water. Calibration was performed using the standard addition method. The results show that ICP-MS allows Pt concentrations to be determined at the level of 10^{-3} µg/l. The instrumental specifications and analytical conditions are reported in Table 2. Fittings of the experimental points were calculated using the routine FORTRAN VA04A program [9].

Table 1 ICP-AES apparatus specifications and analytical conditions for the determination of Pt

Spectrometer	Instruments SA, Jobin-Yvon 24 sequential (france)
Monochromator	Czerny-Turner mounting
Nebulizer	Meinhard pneumatic
RF power	1.0 kW
Auxiliary gas flow	14 l/min
Sheath gas flow	0.2 l/min
Aerosol gas flow	0.35 l/min
Sample feed	0.50 ml/min
Analyte line [Pt(II)]	214.42 nm
Reference line [C]	193.09 nm
Integration period	300 ms

Table 2 ICP-MS apparatus specifications and analytical conditions for the determination of Pt

Spectrometer	Elan 5000 ICP-MS (Perkin-Elmer, USA)
Nebulizer	Cross flow, Scott chamber
RF power	1.0 kW
Plasma gas flow	16 ml/min
Auxiliary gas flow	1 ml/min
Aerosol gas flow	1 ml/min
Sample feed	1 ml/min
Element/mass	Pt/195
Optimization	on signal: ^{103}Rh, ^{24}Mg, ^{208}Pb
Replicate time	15000 ms
Dwell time	300 ms
Sweeps for readings	5
Reading/replicate	3
Scanning mode	Peak hop transient

Results

In all, 31 blood samples from Friday drawings and 18 from Monday drawings were available for the analysis. The drawings covered the 5 weeks of the therapy schedule. Analyses were performed for both total and free Pt, and the element concentration was reported versus the sampling day. Dispersion diagrams are given in Fig. 2.

Relevant differences were observed in Pt levels detected in patients at the same day of therapy; thus, it could be difficult to propose a function for the drug trend during therapy. However, considering that (a) no drug is present at

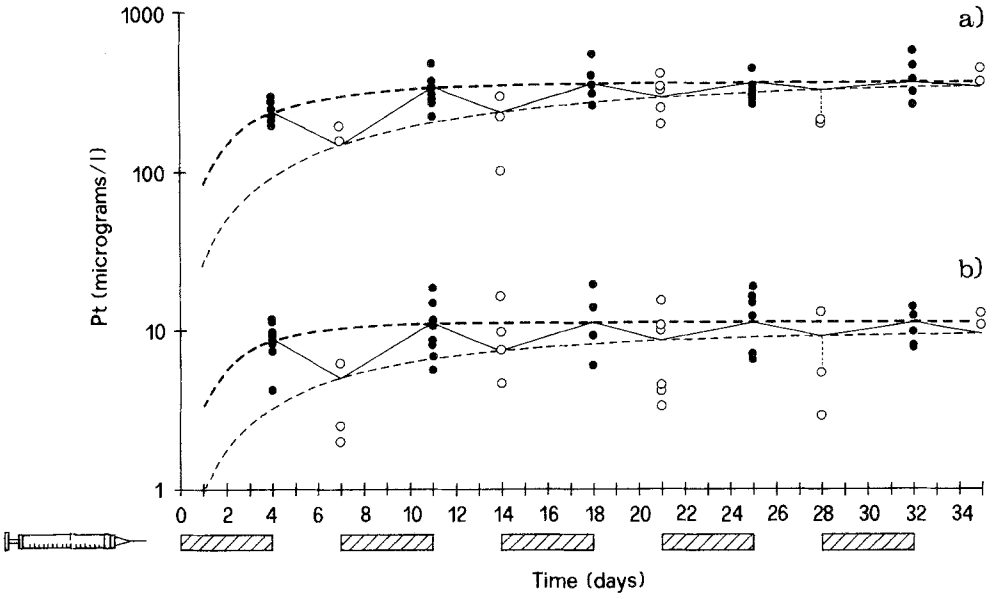

Fig. 2 a, b Plasma concentration levels (μg/l) and best-fitting curves generated for total and free Pt on Fridays and on Mondays reported vs sampling day. Each point represents the Pt concentration for an individual patient. *Solid circles* stand for the concentrations measured on Friday and *open circles*, for those determined on Monday. **a** Best-fitting curves for total Pt on Fridays [(– – –), $R^2 = 0.484$] and on Mondays [(– – –), $R^2 = 0.548$]. **b** Best-fitting curves for free Pt on Fridays [(– – –), $R^2 = 0.244$] and on Mondays [(– – –), $R^2 = 0.262$]

Table 3 Platinum levels determined on Mondays and Fridays during the 5 weeks of infusion

Weeks	Total Pt (μg/l)[a]		Free Pt (μg/l)[a]		Percentage of free Pt vs total Pt	
	Friday	Monday	Friday	Monday	Friday	Monday
1	238 (195–294)	148 (158–209)	8.6 (4.2–11.5)	5.0 (2.0– 6.2)	3.59	3.37
2	341 (218–478)	237 (101–299)	11.0 (5.6–18.1)	7.4 (4.6–16.3)	3.23	3.12
3	356 (260–528)	290 (199–412)	11.2 (6.0–19.2)	8.6 (3.3–15.4)	3.15	2.96
4	358 (265–438)	322 (203–376)	11.2 (6.5–18.5)	9.2 (2.9–13.0)	3.13	2.86
5	358 (265–572)	341 (364–444)	11.2 (7.7–13.8)	9.4 10.8–12.8)	3.12	2.75

[a] Calculated values (the minimum and the maximum of the experimental values are shown within parentheses)

Table 4 Comparison between the present study and selected 5-day CVI reported in the literature

Parameter	5-day CVI		Present study	R[a]	R′[a]
	[4]	[7]			
Number of patients	5	14	22		
Duration of infusion (h)	120	120	500		
Duration of treatment (h)	120	120	840		
CDDP daily dose (mg/m^2)	25	40	4	6.25	10
Total CDDP dose delivered (mg/m^2)	125	200	83	1.51	2.41
Maximal concentration of free Pt (mg/l)	0.138	0.170	0.0110	12.5	15.4
Maximal concentration of total Pt (mg/l)	2.38	3.22	0.358	6.60	8.99
Percentage of free Pt vs total Pt	5.80	5.30	3.10	1.90	1.70

[a] Ratio of parameters: R = [3]/present study; R′ = [7]/present study

the beginning of the treatment and (b) Pt concentrations initially increase rapidly with time and then undergo smaller variations thereafter, it seems to us that representative values for the Pt concentration measured on Friday ($[Pt]_{Friday}$) and on Monday ($[Pt]_{Monday}$) can be calculated by the general expression:

$$[Pt] = K(1\text{-}e^{-kt}),$$

where $[Pt] = [Pt]_{Friday}$ on days 4, 11, 18, 25, and 32 or $[Pt] = [Pt]_{Monday}$ on days 7, 14, 21, 28, and 35 and t = time (in days).

Fittings of the experimental points, reported as dotted curves in Fig. 2, gave values for K, k and for the percentages of explained variance R^2%. The experimental and the calculated platinum levels are summarized in Table 3.

The variations in Pt levels observed during the therapy are schematically represented in Fig. 2 by linking up of the calculated values for $[Pt]_{Friday}$ and $[Pt]_{Monday}$. These curves allow the Pt levels for the tested therapeutic schedule to be compared with literature data for selected 5-day continuous venous infusion (CVI; Table 4). If normalization is made for the total delivered CDDP dose, the long-term infusion reported in the present study yields a strong decrease in the amounts of both free and total Pt detected in plasma (see R and R' values in Table 4). The percentage of free drug versus total drug calculated from the fitting curves was

about 3% and did not vary with the therapy duration. It was comparable with the values found when the same total dose was given over a shorter period (Table 4). Moreover, this percentage remained unchanged after the rest from infusion between Friday and Monday, although considerations of the half-life values for free and total Pt [6] would suggest a decrease in free Pt versus total Pt.

Discussion

The clinical effects of radiochemotherapy with CDDP at low doses have been the subject of a large number of recent investigations [7, 10]. However, the present study is the first attempt to dose CDDP such that it and its metabolites occur at very low concentrations in the plasma of patients. Some of the results were expected; maximal values for both total and free Pt in plasma were very low (about 1 order of magnitude less than the examples reported in Table 4), thus confirming the low level of toxicity of the present administration schedule as regards the effects of CDDP ([2]; Bedini, submitted for publication).

The percentage of free versus total Pt in plasma did not significantly differ from that found in some selected 5-day CVI in which the same total dose was given over a shorter period; moreover, it did not decrease 3 days after the drug administration was stopped. From this result it seems that on the proposed administration schedule, Pt capable of crossing membranes having a cutoff value of 10,000 Da is not free from interaction with plasma proteins, and what is called "free" Pt can be tentatively thought of as Pt bound to low-molecular-weight proteins. Further sampling is in progress to quantitate the protein content.

Finally, it was surprising that the variation in both total and free drug levels due to the 3-day rest from CDDP administration decreased during the therapy. However, considering that at present the percentages of explained variance of the four regression models are low ($R^2\%$ = 48 and 54 for total Pt and 24.4 and 26.6 for free Pt), further selection of patients is necessary if all previous conclusions are actually to be definitely accepted and pharmacokinetic parameters, determined.

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