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Short communication

Tumor-tissue and plasma concentrations of platinum during chemotherapy of non-small-cell lung cancer patients*

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Summary. Tumor-tissue and plasma concentrations of platinum were studied prospectively in two groups of eight patients who were suffering from advanced non-small-cell lung cancer. Treatments including two different schedules of cisplatin administration (25 vs 100 mg/m^2 on day 1) were compared. At 30 min after the beginning of the cisplatin infusion, blood samples and bronchoscopically obtained biopsy specimens were taken for determinations of platinum concentrations by means of flameless atomic absorption spectrophotometry. The procedure did not induce any complication. Total plasma platinum concentrations at 30 min were significantly lower (P <0.01) in patients receiving 25 mg/m^2 (0.49 ± 0.23 μg Pt/ml) than in those receiving 100 mg/m^2 (1.44 ± 0.62 μg Pt/ml), whereas no significant difference was observed in tumor-tissue platinum concentrations (22.49 ± 53.89 ng Pt/mg in patients receiving 25 mg/m^2 vs 51.13 ± 65.52 ng Pt/mg in those receiving 100 mg/m^2). There was a weak correlation between simultaneous plasma and tumor-tissue platinum concentrations at 30 min. Tumor-tissue platinum concentrations seem to be poorly influenced by the cisplatin dose. This finding suggests a great interindividual variability of platinum tumor-diffusion properties in non-small-cell lung cancer.

Introduction

Advanced non-small-cell lung cancer (NSCLC) requires combined modality treatment because of early relapses following surgery alone. However, NSCLC has poor sensitivity to chemotherapeutic agents [6, 10]. Treatments including *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP) give an objective response rate of nearly 40%, which is closely related to the performance index and the stage of disease, two well-known prognostic factors [7, 9, 11]. However, little is known about the tumor-tissue concentration of chemotherapeutic agents, although it may be presumed to be a critical factor influencing the efficacy of chemotherapy. A spectrophotometric dose of total plasma platinum has been proposed in the management of chemotherapeutic toxicity [4, 8]. Moreover, tumor-tissue concentrations of platinum have been studied in head and neck tumors [3, 5], uterine cervix carcinomas and breast carcinomas following CDDP infusion [5].

In a prospective study, two groups of patients suffering from advanced NSCLC were treated by two different schedules of CDDP administration. During the first infusion, the plasma concentration of platinum was assessed in parallel with the tissue concentration in tumor biopsy specimens obtained by fiber-optic bronchoscopy. This study was undertaken to determine (1) whether the assessment of tumor-tissue concentrations of platinum is possible and safe, (2) whether there is a relationship between plasma and tumor-tissue concentrations of platinum, and (3) whether the CDDP dose influences plasma and tumor-tissue platinum concentrations.

Patients and methods

Patients

A total of 16 patients (15 men and 1 woman aged 42–73 years; range, 59.6 ± 9.6 years) suffering from NSCLC were included in the study. All lung tumors were analysed according to the latest WHO classification [17] by light microscopy following hemtaoxylin-eosin staining of biopsy specimens. Among the tumors were ten squamous-cell carcinomas, three adenocarcinomas and three large-cell carcinomas. Staging of NSCLC was performed according to the 4th edition of the IUCC tumor classification [13]. This evaluation disclosed 6 patients with stage III A disease, 2 with stage III B disease and 8 with stage IV disease (Table 1).

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Table 1. Patients and clinical data

CDDP dose	Patient number	Age (years)	Histology	TNM[a]	Stage	PS
100 mg/m²	1	61	SQC	T3N2M1	IV	3
	2	46	SQC	T3N2M1	IV	2
	3	60	SQC	T3N3M1	IV	2
	4	48	Ade	T2N1M1	IV	1
	5	63	SQC	T4N2M1	IV	3
	6	70	SQC	T3N2M0	IIIA	2
	7	65	SQC	T4N3M0	IIIB	2
	8	57	SQC	T4N3M0	IIIB	2
Mean ± SD		58.7± 7.6				2.1±0.6
25 mg/m²	9	71	SQC	T3N2M0	IIIA	1
	10	67	LCC	T3N3M1	IV	2
	11	60	LCC	T3N2M0	IIIA	2
	12	42	Ade	T3N2M1	IV	2
	13	73	Ade	T3N2M0	IIIA	1
	14	45	LCC	T3N2M0	IIIA	1
	15	62	SQC	T3N2M0	IIIA	2
	16	65	SQC	T3N3M1	IV	3
Mean ± SD	60.6±11			1.75±0.7		

[a] According to the 4th edition of the UICC classification [13]. SOC, squamous-cell carcinoma; Ade, adenocarcinoma; LCC, large-cell carcinoma; PS, performance status

Treatments

Two doses of CDDP were compared (Table 1). In each protocol, patients received the drug (Roger Bellon, Levallois Perret, France) diluted in a 0.9% NaCl solution that was given as a short intravenous infusion over 2 h. In protocol A ($n = 8$), CDDP was given at a dose of 100 mg/m² on day 1 of treatment; the associated drug was 5-fluorouracil, which was given on days 1–5 (phase II trial of CDDP and 5-fluorouracil in squamous-cell and large-cell carcinomas of the lung [12]). In protocol B ($n = 8$), CDDP was given at a dose of 25 mg/m² from day 1 to day 4 of treatment; the associated drugs were ifosfamide and etoposide, which were given on days 1–4 (pilot study of primary chemotherapy in unresectable NSCLC). Both studies, controlled by the Fédération Française des Centres de lutte contre le Cancer, were carried out in our institution.

In the two therapeutic schedules the CDDP infusion was given from 6:00 to 8:00 p.m. so as to avoid circadian variations in the toxicity and elimination of the drug. Only the first dose infused on day 1 (25 or 100 mg/m²) was taken into account by the study, since the assessment of tumor-tissue and plasma platinum concentrations was done during the 1st day of treatment. Moreover, the associated drugs were given after the first infusion of CDDP to avoid pharmacological interactions. Hyperhydration consisted of the infusion of 4 l NaCl solution daily, avoiding mannitol. There was no significant difference between the two groups of patients when age, TNM classification, or performance status were considered (Table 1).

Fiber-optic bronchoscopy

Bronchoscopy was performed with a flexible fiber-optic instrument (Olympus BF type 1T10) at 15 min after premedication consisting of 0.25 mg atropine and local anaesthesia with 15 ml 1% lidocaine. The bronchial tumor biopsies were done with forceps (FB-20C), and from three to five specimens (30–60 mg wet tissue) were taken from the same site and pooled in a glass tube in dry air until the platinum was titrated. When the volume of the biopsies was sufficient, they were divided into two groups for assessment of the intra-tumoral variability of tumor-tissue platinum concentrations.

Platinum assay

Platinum was determined in plasma and tumor-tissue specimens by means of flameless atomic absorption spectrophotometry [4, 8] using a Varian 1275-AA spectrophotometer (Varian SA, Walnut Creek, USA). The sample size was 10 µl and an autosampler was used. Determinations were based on at least three injections of each sample.

Total platinum plasma determination. Samples of plasma were stored at 4°C until platinum determination. Briefly, adjustment of the dose was assessed by a standard curve plotted using five concentrations of platinum between 25 and 250 mg/ml for every third dose. The lower limit of quantitation was 0.025 µg Pt/ml and the coefficient of variation for triplicate samples was <1%. The reproducibility of the assay gave a variation of <2%.

Tumor-tissue platinum determination. Tumor specimens were air-dried to constant weight for 8 days. The dry weight of specimens ranged from 1.5 to 5 mg/biopsy. Nitric digestion was performed in 65% HNO_3, and 0.5-ml samples were analysed for platinum determination, as were the plasma samples, by means of flameless atomic absorption spectrophotometry [3, 5, 8]. Results were expressed as ng Pt/mg tissue.

Study design

All patients had a PaO_2 value of >70 mmHg and none of them had received either chemotherapy or radiation treatment prior to testing. Before the first infusion of CDDP, a blood sample (t = 0 min) was drawn by puncture of a forearm vein; then, CDDP infusion was begun at a dose of either 25 or 100 mg/m². An auto-syringe infusion pump was used to maintain a constant drug-infusion rate. At 30 min after the beginning of the infusion, the flow rate was checked to ensure the delivery of 25 and 6.25 mg/m² in protocols A and B, respectively, and a new blood sample was taken from the forearm opposite the one receiving chemotherapy. Fiber-optic bronchoscopy was done such that biopsies could be performed in a non-necrotizing area of the bronchial primary tumor at 30–35 min after the beginning of the CDDP infusion.

To ascertain that platinum determinations were done on malignant specimens, a routine pathological examination was performed on cytological prints of the biopsies in six cases and on additional biopsies taken from the same site in the other cases after hematoxylin-eosin staining. The study was approved by the Montpellier University Ethical Committee, and informed consent was obtained from each patient; in particular, the study was explained to them as being investigational and not necessary for clinical care.

Statistical analyses were done by means of non-parametric tests. Differences between groups were determined using the Mann-Whitney U-test, and $P < 0.05$ was considered to be significant; Spearman rank-order correlation coefficients were calculated.

Results

Fiber-optic bronchoscopy

Depending on the localisation of the tumor, biopsies were taken from a main bronchus in 3 cases and from a lobar bronchus in 13 cases. No complication was observed during or after fiber-optic bronchoscopy; in particular, we observed no hemoptysis or respiratory function impairment. The total duration of the fiber-optic procedure did not exceed 10 min. In all cases, routine pathological examination of the cytological prints of the biopsies or of additional biopsies demonstrated the presence of malignant cells.

Table 2. Global results of platinum TITRATION

CDDP dose	Patient number	Pl(t0)μg/ml	Pl(t30)μg/ml	Tu(t30)ng/mg mean (range)
100 mg/m²	1	<0.025	2	195
	2	<0.025	1.07	58.60
	3	<0.025	0.46	14.60
	4	<0.025	2.34	12.50
	5	<0.025	1.24	11 (10.6–11.4)
	6	<0.025	2.13	115.40
	7	<0.025	0.83	1
	8	<0.025	1.42	0.98
25 mg/m²	9	<0.025	0.35	3 (2.70–3.30)
	10	<0.025	0.70	1.66
	11	<0.025	0.32	0.50
	12	<0.025	0.26	0.50
	13	<0.025	0.52	0.25
	14	<0.025	1.02	165
	15	<0.025	0.40	6 (5.5–6.5)
	16	<0.025	0.39	3

Pl, total plasma concentration of platinum; Tu, tumor-tissue concentration of platinum

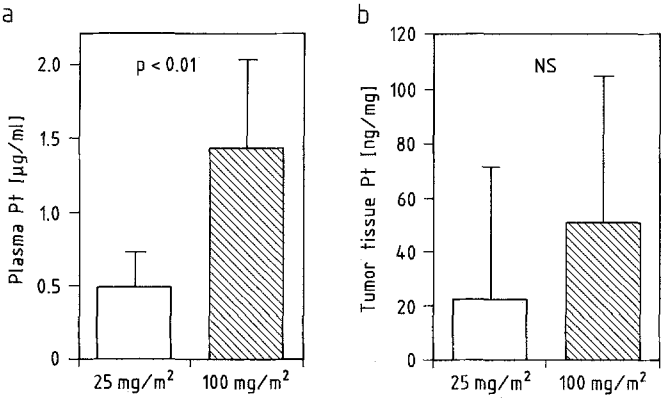

Fig. 1 A, B. Comparison of **A** plasma total platinum concentrations and **B** tumor-tissue platinum concentrations between patients receiving 25 mg/m² CDDP (*open bars*) and those receiving 100 mg/m² (*cross-hatched bars*). Values represent the mean ± SD. Statistical analyses, Mann-Whitney *U*-test

Plasma platinum determination

Total plasma concentrations at t = 0 min were <0.025 ng Pt/ml in all cases. The total plasma concentrations at t = 30 min differed significantly according to the delivered dose of CDDP (P <0.01, Mann-Whitney U-test): the total plasma platinum concentration was lower in patients receiving 25 mg/m² (0.49 ± 0.23 μg Pt/ml) than in those receiving 100 mg/m² (1.44 ± 0.62 μg Pt/ml; Table 2, Fig. 1).

Tumor-tissue platinum determination

Tumor-tissue platinum determination was done in all cases. In three cases it was performed separately on two groups of biopsies. The variation in tumor-tissue platinum concentrations did not exceed 1 ng Pt/mg dry specimen. In these cases, the mean tumor platinum concentration was taken into account.

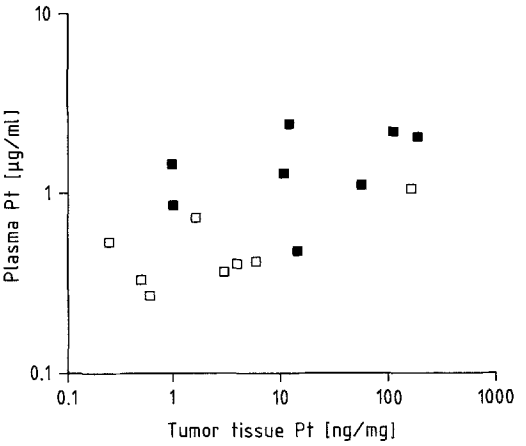

Fig. 2. Relationship of log plasma total platinum concentrations to log tumor-tissue platinum concentrations (n = 16; r_s = 0.57, P <0.05). *Open squares*, patients receiving 25 mg/m² CDDP; *closed squares*, patients receiving 100 mg/m²

A great intersubject variability in tumor-tissue platinum concentrations was seen, ranging from 0.25 to 195 ng Pt/mg dry specimen. No significant difference was observed in tumor-tissue platinum concentrations when the delivered dose of CDDP was taken into account, although levels were lower in patients receiving 25 mg/m² (22.49 ± 53.89 ng Pt/mg) than in those receiving 100 mg/m² (51.13 ± 65.52 ng Pt/mg; Table 2, Fig. 1). There was a weak correlation between plasma and tumor-tissue platinum concentrations (r_s = 0.57, P <0.05; Fig. 2).

Discussion

This study demonstrates that the assessment of tumor-tissue platinum concentrations is possible by analysis of lung-cancer specimens procured by fiber-optic bronchoscopy. At t = 30 min, a significant difference in plasma platinum concentrations between the two groups was observed, depending on the delivered dose of CDDP, whereas simultaneous tumor-tissue platinum concentrations seemed to be poorly influenced by the drug dose. There was a weak correlation between simultaneous plasma and tumor-tissue platinum concentrations at t = 30 min.

Fiber-optic bronchoscopy biopsies of lung cancer give valid tumor-tissue specimens that are available not only for routine histology but also for immunohistology [2] and DNA-marker analyses [15]. In our study, we used bronchoscopically procured lung-cancer specimens to assess tumor-tissue platinum concentrations. This procedure is safe and does not provoke any major discomfort when it is performed during the CDDP infusion. Digestive toxicity is not increased by fiber-optic bronchoscopy; however, it must be emphasized that this procedure requires both a proximal tumor that is directly accessible by means of the fiber-optic instrument (lobar or main bronchus tumor) and a PaO_2 of >70 mmHg. Moreover, a routine histology of the

specimens is needed to certify that the biopsy specimens involve tumor tissue.

Plasma platinum determination has been proposed in the management of solid tumors because of dose-dependent CDDP toxicity [1, 16]. It has been shown that the maximal plasma non-filterable (non-protein-bound) platinum level is 10–40 times lower for extended 5-day infusions than for a short-term infusion [1]; on the other hand, the area under the filterable platinum curve is greater for the extended infusion than for the short-term treatment. The present study demonstrates that different modalities of infusion of the same CDDP dose result in different plasma platinum concentrations and different levels of urinary platinum excretion. However, it is not well established as to whether or not the modality of CDDP administration influences the tumor platinum concentration.

Recently, flameless absorption spectrophotometry has been used to assess the tumor platinum concentration in nitric digested specimens from various sites [3, 5]. In all, 24 head-and-neck carcinomas were treated with 50 mg/m^2 CDDP on days 1 and 2 and biopsies were analysed on day 3 for their platinum content [3]. This study disclosed a great interindividual variation in tumor-tissue platinum concentrations. More recently, platinum concentration has been determined in autopsy tumor samples from patients who had received various doses of CDDP [14]. Platinum concentrations in brain gliomas and metastases were higher for patients whose tumors had responded to CDDP-containing regimens than for those whose lesions had not responded; the tumor platinum concentration correlated with the cumulative CDDP dose and depended on the time since the last treatment.

In the present study, the plasma platinum concentration was significantly lower in patients receiving 25 mg/m^2 than in those receiving 100 mg/m^2. This finding suggests that the plasma concentration may be dependent on the schedule of administration of the same dose (100 mg/m^2 on day 1 vs 25 mg/m^2 on days 1–4). However, NSCLC tumor-tissue platinum concentrations do not differ significantly according to the CDDP dose. Fiber-optic bronchoscopy was done 30 min after the beginning of the drug infusion to ensure optimal patient conditions, owing to late CDDP emetic toxicity. For this reason, we cannot exclude the possibility that variations in tumor-tissue platinum concentrations may occur during later phases of the CDDP infusion. However, intersubject variability in tumor-tissue platinum concentrations suggests a great heterogeneity of platinum tumor-tissue diffusion properties. Progress in anti-emetic therapy and the safety of the procedure described should enable further studies to determine later platinum concentrations in bronchoscopically obtained lung-tumor biopsies.

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