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CLINICAL, ANALYTICAL AND PHARMACOKINETIC ASPECTS IN CANCER CHEMOTHERAPY WITH PLATINUM COORDINATION COMPOUNDS

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Summary. - *A survey of investigations performed by our group over the last few years whose goal was to obtain analytical, clinical and pharmacokinetic data concerning cancer chemotherapy with Pt-based drugs is reported. From this standpoint the use of inductively coupled-plasma atomic emission spectrometry for determining Pt levels in biological samples is discussed, particularly as regards: 1) the amelioration of sample introduction procedures into the torch in the case of micro-sampling and 2) the investigation of plasma drug distribution by means of liquid chromatography techniques. Clinical studies evaluated therapeutic response and toxicity during regional and systemic treatments with Cisplatin and Carboplatin against solid tumors in adults as well as in children. Several pharmacokinetic parameters such as plasma half-lives for free and protein-bound drug, tissues exposure as determined by AUC and urinary excretion are examined.*

Riassunto (Aspetti clinici, analitici e farmacocinetici nella chemioterapia antitumorale a base di composti di coordinazione del platino). - *Sono riportati i risultati di ricerche eseguite durante gli ultimi anni allo scopo di ottenere parametri analitici, clinici e farmacocinetici relativi alla chemioterapia antitumorale con farmaci a base di platino. E' presentata l'applicazione della spettrometria di emissione atomica a plasma induttivo alla determinazione del platino in campioni biologici, con particolare attenzione a: 1) ottimizzazione delle procedure di introduzione del campione nella zona di analisi nel caso di microcampionamenti e 2) studio della distribuzione del farmaco nel plasma previa separazione mediante cromatografia liquida. L'indagine clinica ha preso in esame la risposta terapeutica e la tossicità in trattamenti sistemici e regionali con Cisplatino e Carboplatino nel caso di tumori solidi di pazienti adulti e in età pediatrica. Sono stati infine valutati parametri farmacocinetici quali l'emivita plasmatica del farmaco cosiddetto libero e legato alle plasmaproteine, l'esposizione dei tessuti espressa in termini di AUC e l'escrezione urinaria.*

Introduction

Studies concerning the biological properties of platinum coordination compounds and their application in cancer chemotherapy began in the late 1960s, when antitumor activity of Cisplatin (cis-diamminedichloro platinum II, CDDP) against rodent tumors was first reported [1]. Since its introduction in clinical practice, this compound has proved to be one of the most effective antiproliferative drugs in the treatment of a wide range of human malignancies. Although therapeutic activity of Cisplatin appeared to be dose-dependent, several toxic effects limited its clinical usefulness. Most of these side effects were already noted in the early phase I trials: nausea and vomiting, myelosuppression, hearing impairment, peripheral neuropathy and renal damage, this last being identified as the dose-limiting factor. Subsequent studies showed that diuresis induced by intensive parenteral hydration and diuretics partially prevented renal toxicity [2]. Administration of Cisplatin in hypertonic saline with intensive parenteral hydration in combination with diuretics further ameliorated nephrotoxicity, allowing the dose to be increased up to 200 mg/m^2/course. Nevertheless, neurotoxicity and myelosuppression appeared to be dose-limiting effects with this high-dose rapid (< 1 h) intravenous (*i.v.*) infusion regimen [3].

Since previous *in vitro* studies demonstrated that prolonged exposure of tumor cell lines to Cisplatin results in a cytotoxic effect equivalent to short exposure at an order of magnitude greater than drug levels [4], a number of studies employing continuous i.v. infusion of Cisplatin at conventional doses (≤ 120 mg/m^2/course) were performed [5, 6]. In this framework, the studies reported here are aimed at evaluating the feasibility of a schedule combining Cisplatin at high doses with prolonged (5 days) continuous infusion administration. Gastrointestinal toxicity, nephrotoxicity and neurotoxicity were lessened by this regimen, myelosuppression remaining the only dose-limiting factor [7]. As an alternative to the clinical approach referred to

above, the antitumor activity of Cisplatin was also exploited on limb tumors in isolated hyperthermic perfusion regimen [8, 9]. In this case, systemic toxicity was lessened, while local toxicity remained the dose-limiting factor.

At the present stage of clinical research, an improved therapeutic index can derive only from the identification of less toxic second-generation Cisplatin analogues possessing equivalent or even higher antitumor activity. Up to now sufficient experience has been gained to be confident about the therapeutic utility of only Carboplatin (cis-diammine-1,1-cyclobutane dicarboxylate platinum II, CBDCA). Phase I studies demonstrated that this drug does not induce significant gastrointestinal toxicity, nephrotoxicity and neurotoxicity, while myelosuppression appears to be the only dose-limiting factor, at least in adults, up to doses as high as 1200 mg/m^2/course [10].

A better understanding of the pharmacokinetics and pharmacodynamics of drugs is conditioned by the availability of reliable analytical techniques by which the pharmaceutical agent and related compounds can be quantitated both in various physiological compartments and at different phases of treatment. From this point of view, measurements of the Pt-based agents in biological fluids and tissues were performed by means of a variety of techniques allowing either the determination of the amount of total Pt or of the identification and quantification of different Pt-species.

The first group includes techniques such as radiochemical labelling of the dose [11], neutron activation analysis (NAA) [12], differential-pulse polarography (DPP) [13], X-ray fluorescence (XRF) [14], mass spectrometry (MS), and electrothermal atomization atomic absorption spectrometry (ETA-AAS) [12, 13]. Although adequate in some instances, each of these methods has its own shortcomings such as too short half-lives of Pt radionuclides, poor sensitivity, restricted linear concentration range, matrix problems, time consuming sample pretreatment and undue patient stress.

Platinum-based drugs currently under study show a trend to irreversibly bound to intra- and extracellular proteins as well as to DNA. Since only the so-called free drug can diffuse into the cells, the formation of adducts with extracellular macromolecules, mainly plasma proteins, prevents a large amount of the element from interacting with DNA. Thus, as regards pharmacokinetic evaluation, the simple determination of total Pt plasma levels is not a reliable indication of the relationship linking tissue exposure with therapeutic response and toxicity. Consequently, from an analytical standpoint, the second group procedures, aimed at discriminating and quantifying the various Pt compounds which form *in vivo*, are more powerful techniques for clinical investigation. To date, the most suited and promising analytical systems for this purpose are based on determination of different species after high performance liquid chromatography (HPLC) separation [14-17]. The conventional non-destructive UV detection can in such cases be combined with more specific and sensitive systems to quantify the elemental species in each eluted fraction. Use has been made so far of on-line or off-line determination by flame AAS, ETA-AAS, ED, MS and inductively-coupled plasma atomic emission spectrometry (ICP-AES).

In this last instance our group has recently undertaken the further development and application of ICP-AES to the above clinical problem [18]. ICP-AES is known to possess general features as accuracy, precision and sensitivity. Furthermore, this technique is highly flexible and therefore apt to solve specific analytical problems occurring in Pt quantification for the monitoring of antitumoral Pt-based agents. The achievements attained so far in this research area are detailed hereafter.

Experimental approach

Clinical and pharmacokinetic studies

The steep dose-response curve observed in *in vitro* systems [4, 19] and the dose-response relation demonstrated in clinical studies [20], suggested that for Cisplatin the increase beyond conventional doses (100-120 mg/m^2/course) could produce a greater therapeutic effectiveness in more patients. Such dose escalation was performed by Ozols: nevertheless, irreversible neurotoxicity remained a dose-limiting factor [3]. Insofar as Cisplatin is concerned, previous authors seem to agree almost unanimously that the adverse effects induced by this drug could be attenuated by administering the drug by *i.v.* infusion for prolonged periods of time [5, 6]. Thus, a multifaceted study was planned in order to investigate more systematically the toxicity and the therapeutic efficacy of Cisplatin when administered at high doses by continuous *i.v.* infusion in combination with etoposide (VP-16-213) [7].

Eligibility criteria required patients to have: a) histologically confirmed malignant solid tumor; b) unequivocal disease; c) life expectancy greater than six weeks; d) adequate blood cell counts, i.e. leukocyte count > 4000/μl and platelet count 150,000/μl; e) normal liver function, i.e. serum bilirubin < 1.0 mg/dl and SGOT < 30 U/l; f) normal renal function, i.e. blood urea nitrogen (BUN) < 20 mg/dl and serum creatinine < 1.0 mg/dl; and g) recovery from any previous treatment. Informed consent was obtained from the patients or their parents.

Twenty-one patients with malignant solid tumors ranging in age from 10 months to 18 years (median 3 years) were enrolled in this study. Thirteen of these patients failed previous treatment: two patients had radiotherapy, four had chemotherapy and seven were given both radiotherapy and chemotherapy. Five patients had also received Cisplatin at conventional doses.

The daily dose of Cisplatin (40 mg/m^2) was diluted in normal saline (3 l/m^2). Twenty meq/l of KCl, 3.5 ml/l of 10% $CaCl_2$ and 1.5 ml/l of 50 % $MgSO_4$ were added to the infusion liquid, which was then infused continuously over 24 hours. The daily dose of etoposide (100 mg/m^2) was diluted in 100 ml of normal saline and infused for one hour.

Treatment was given for five consecutive days. Intensive parenteral hydration was also given one day before and one day following treatment. Standard diuretic and antiemetic therapy was also administered. Courses were repeated every 28 days. Toxicity was evaluated according to the Eastern Cooperative Oncology Group (ECOG) criteria [21]. Seventy-one courses were performed, with a median of four courses per patient.

Another research line, centered on Cisplatin administration, was devoted to the exploitation of the drug activity when combined with hyperthermal treatment of limb tumors. Thirty patients were treated with hyperthermic antiblastic perfusion (HAP) using Cisplatin at different dosages injected either by bolus or by four fractionated doses. A temperature of about 41.5 °C was achieved in the muscle and tumor [8, 22].

At a later stage of the program we decided to evaluate whether another Pt-based agent, Carboplatin, could safely replace Cisplatin in an etoposide-containing regimen. Initially, à daily dose of 300 mg/m² was administered by continuous *i.v.* infusion over a two day period in association with pulsed etoposide at the dose of 100 mg/m²/day on days 1, 2 and 3. Even though only two patients were treated at this dose level, and thus general conclusions cannot be drawn, toxicities were found to be extremely mild: gastrointestinal toxicity, nephrotoxicity and neurotoxicity were not observed, mild myelosuppression being the only encountered adverse effect. Based on the aforementioned findings, we have increased the dose of Carboplatin from 300 to 500 mg/m²/day, maintaining the 24 hour infusion. Since the adverse effects were still moderate, we reduced the duration of administration from 24 to 5 hours. Courses were repeated every 21 days up to a maximum of four.

Eligibility as well as toxicity and response criteria, were as previously reported (see Cisplatin). Twenty-three patients with malignant solid tumors have been entered up to now in this protocol, ranging in age from 5 months to 16 years (median 6 years). Eleven patients failed to respond to previous radio- and/or chemotherapy: seven of them had also received Cisplatin at high doses. A total of 67 courses were performed, with a median of 3 courses per patient.

Finally, the evaluation of pharmacokinetic parameters for Pt-containing antitumor agents in repeated courses was carried out quantitating Pt species in biological fluids (plasma, cerebrospinal fluid and urine) and bioptic tumor samples. While total Pt levels were determined throughout the study by means of ICP-AES, Pt species were separated first by centrifugal ultrafiltration (cut-off values: 50,000 and 10,000 Da) during preliminary studies, and later by means of an HPLC system in successive investigations.

Analytical procedures

The main information on ICP-AES instrumentation employed and working conditions adopted are summarized in Table 1. At pre-established times, as dictated by the protocol adopted, urine, plasma and tissue samples were drawn, immediately frozen and stored at - 20 °C until analysis. All biological fluids were analyzed without any pretreatment except for dilution with doubly-distilled water. Tissue samples were dry ashed at 450 °C for 12 h in a quartz-lined muffle furnace and ashes were dissolved in 2% (v/v) HNO_3.

Table 1. - *Operating conditions for ICP-AES analysis*

Spectrometer	Jobin-Yvon 38 VHR
RF generator	DURR-JY 3848, frequency 56 MHz, nominal power output 2.2 kW
Torch	INSA, demountable with 6 coils (o.d. 32 mm, height 30 mm), with plasma argon flow 18 l/min· coating argon flow 0.3 l/min and aerosol argon flow 0.45 l/min
Monochromator	Hr 1000 M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic plane grating, linear dispersion in the first order 0.27 nm/mm, theoretical resolution 504,000, spectral range 170-450 nm, entrance and exit slit width: 40 μm
Polychromator	HR 1000 M, focal length 0.5 m, Paschen-Runge mounting, equipped with a 3600 grooves/mm holographic concave grating,entrance and exit slit width: 50 μm
Spectral line	Pt (II) 214.42 nm and Pt (I) 265.94 nm
Sample feed	1 ml/min

As a rule, calibration curves were preferably obtained by adding Pt aliquots to the solutions originating from different matrices, even though the analytical technique tolerates the calibration in aqueous media, with a relative standard deviation (RSD) within 5%. Typical precision ranged between 0.9-2.5% in biological samples with Pt levels of 0.2-5.0 mg/l, i.e. within the concentration interval of clinical importance. Due to lack of appropriate reference materials for the investigated Pt compounds, the accuracy of the method was estimated according to the established addition procedure. At clinical concentrations, percentage recovery values were found to range between 98.5 and 101.7. As regards the dynamic concentration range, linear responses were observed at least for four orders of magnitude. Detection limits, calculated on the basis of the 2σ criterion, were of about 10 μg/l and are thus more than adequate for purposes outlined above.

The analytical capabilities of the ICP-AES technique, are hampered by the fact that conventional systems of introducing liquid samples into the torch, such as pneumatic and ultrasonic nebulization devices, need amounts of solution in the ml range. This fact makes their use rather problematic when frequent blood sampling and biopsies are required, especially if the Pt levels are very low. Among the various attempts to overcome this drawback, the electrothermal vaporization (ETV) appears to be the

most promising approach to deal with microvolumes of sample which can thus be directly conveyed into the torch as atomic vapours and not as sprayed solutions [23, 24]. Briefly, a conventional electrothermal graphite atomizer for AAS was connected to an ICP spectrometer using an electrically heated quartz tube. Table 2 lists the operating conditions and thermal programs adopted for Pt determination with the ETV system.

The calibration curves for water, plasma, urine and mineralized tissues, as shown in Fig. 1, appear satisfactory as regards linearity. The matrix influence (probably related to changes in viscosity and/or density of sample solutions), contrary to what was ascertained with the conventional ICP nebulization, can therefore not be disregarded and different plots must be used for each kind of specimen. The graph obtained in water is in fact an adequate standard curve only for undiluted plasma ultrafiltrate.

Some aspects of the analytical performances of the ETV-ICP-AES combination are reported in Table 3. The precision (% RSD) ranged from 4 to 7% at 50 µg/l and the accuracy (evaluated as recovery) was 103 to 105%. The detection power was found to be 0.25-0.5 µg/l (25-50 pg) depending upon the matrix. The latter values are one order of magnitude better than those obtained using conventional nebulizers. Furthermore, as concerns the dynamic concentration range, the typical linear response of ICP technique was maintained in the ETV-ICP-AES version. This fact makes Pt analyses even more flexible and expedient in monitoring new therapeutic regimens for which the sample amounts are often very limited.

To solve the problem of simultaneous assays of total Pt and various Pt compounds possibly present in plasma, separation through a centrifugal ultrafiltration procedure becomes mandatory, as suggested by Bannister *et al.* [25]. Aliquots of 2 ml of plasma were centrifuged at 5,000 x *g* for 1 h at 4 °C in microconcentrators, having cut-off values of 50,000 and 10,000 Da, respectively. This procedure allows the amount of complexed Pt to be calculated by the difference between the one in plasma and the one in ultrafiltrate fraction.

However, it should be stressed that this approach cannot provide more specific information about the distribution of Pt among the various plasma proteins or about the nature of low molecular weight compounds in which Pt can be present in the ultrafiltrate. Thus, it was compulsory to develop a more adequate analytical method able to simul-

Table 2. - *Operative conditions for thermal vaporization of different matrices in Pt determination by ICP-AES*

Graphite furnace	Perkin-Elmer HGA 400
Injection volume	100 µl
ETV Argon gas flows	carrier: 1.4 l/min, coating: 0.3 l/min

Thermal programs

Sample	Step	Temperature (°C)	Ramp (s)	Hold (s)
water and ultrafiltrates	dry	120	30	10
	ash	1400	20	10
	vaporize	2700	1	5
serum (1+3), urine (1+4) and digested tissue	dry	120	40	20
	ash	1400	30	10
	vaporize	2700	1	5

Table 3. - *Figures of merit for the analysis of Pt in various media by ICP-AES-ETV*

Medium	Detection limit (µg/l)	Calibration plots	RSD (%) C_1	C_2	C_3
Serum (1+3)	0.50	C = - 2.1 + 0.55 I (r = 0.9998)	17	4	5
Urine (1+4)	0.50	C = - 5.2 + 0.47 I (r = 0.9981)	13	7	3.7
Water and ultrafiltrates	0.25	C = 0.65 + 0.55 I (r = 0.9997)	93	4	
Mineralized tissue	0.50	C = - 3.6 + 0.43 I (r = 0.9951)	5	4	5

C = concentration; I = absolute intensity; r = correlation coefficient; C_1 = 10 µg/l; C_2 = 50 µg/l; C_3 = 100 µg/l

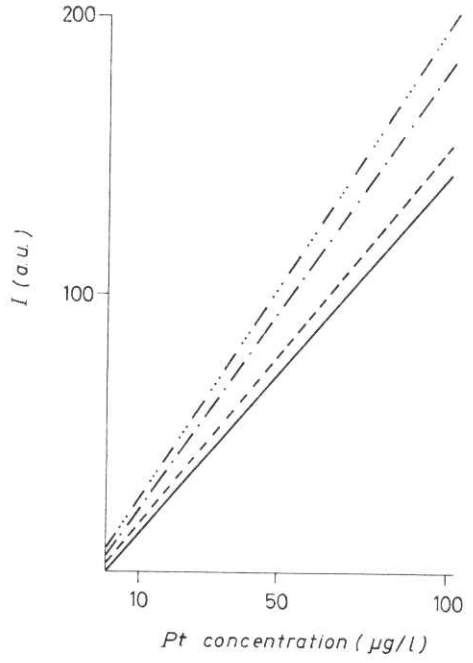

Fig. 1. - Calibration straight lines for Pt determination in biological samples by ETV-ICP-AES: —— water; - - - - 1+3 aqueous serum; - · - · - 1+4 aqueous urine; ····· digested tissue.

taneously separate and quantify the different protein species to which Pt is bound as well as the free aliquot of the element. This was obtained by appropriately coupling the ICP-AES and HPLC techniques.

Regarding the HPLC system, the use of a preparative column was found to be unavoidable because up to 2 ml of plasma had to be injected to nebulize a sufficient amount of the sample into the torch. It should be noted that the chromatograph is directly connected to the spectrometer so that the eluted fractions are sent on-line to the detector. The specific working conditions chosen to perform the analyses and reference standards needed for calibrating the columns are listed in Table 4. The nature and purity of the various protein fractions were further confirmed by immunoelectrophoresis.

The combined technique adopted was characterized by a linear dynamic range for element analysis of 0.2-20.0 µg, an absolute detection limit as low as 38 ng (2σ criterion) and precision of ± 2.5% (at level of 2 µg of Pt). Recovery trials were also more than satisfactory affording amounts as high as 99% of those injected for both drugs in physiological media. Fig. 2 shows the correspondence between the UV chromatographic profile of the eluted proteins and the pattern of ICP-AES detection of Pt contained in the corresponding fractions.

Table 4. - *Operative parameters of the HPLC when combined with the ICP-AES analytical system (for ICP-AES see Table 1)*

Pump	Waters model 510 (USA)
Injector	U 6 K
Column	Pharmacia, Superose 6 and 12 preparative grade, HR 16/50 (Sweden)
Detector UV	Waters model 450, variable wavelength (USA)
Recorder	Perkin-Elmer model LC-100 integrator (USA)
Solvent	Phosphate buffer at pH 6.8 containing 0.07 M KH_2PO_4, 0.1 M NaCl and 6×10^{-4} M NaN_3
Flow	1.0 ml/min
UV wavelength	280.0 nm
Calibrants	Solutions of aldolase, catalase, cytocrome-c, chymotripsinogen, dextran blue, ferritin myoglobin, DNP-L-alanine and ovoalbumin

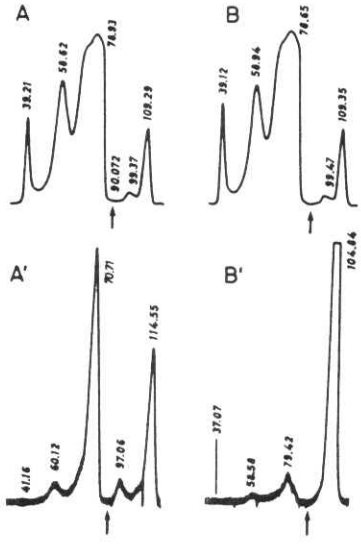

Fig. 2. - Plasma chromatograms of proteins (A, B) and related spectroscopic patterns of Pt (A' for Cisplatin and B' for Carboplatin). The arrows indicate the 10,000 Da cut-off.

Results

Cisplatin

All admitted patients were tested for toxicity and response, and no drug-related death occurred as consequence of treatment. The main adverse effects are listed in Table 5, part a. Gastrointestinal toxicity, nephrotoxicity and neurotoxicity were moderate and acceptable, while the only dose-limiting factor remained transitory even though severe myelosuppression. Moreover, myelosuppression appeared to be cumulative. The median nadir leukocyte count decreased from 1200/μl following the first course to 700/μl after the fourth course. Likewise, the median nadir platelet count was 40,000/μl following the first and halved after the fourth course. Recovery of the leukocyte and platelet counts were also delayed with repeated courses. As regards therapeutic results (see Table 5, part b), in the 13 pretreated patients an overall response rate of 8/13 (62%) was obtained, with one patient achieving complete response. The median duration of these responses was 3 months (range 2 to 5). In the 8 previously untreated patients, the overall response rate resulted 8/8 (100%) with a median duration of 6 months (the range is up to date 2 to 17). For the five Cisplatin-pretreated patients, one complete and four minor responses were obtained.

In all courses, during the infusion of Cisplatin, the average Pt concentrations in plasma (total Pt) and ultrafiltrate at 10,000 Da (free Pt) increased from a minimum at the 24th hour to a maximum at the 120th hour, to give place to a progressive decrease until the beginning of the subsequent course (Fig. 3). The present findings confirm that plasma Pt levels decay according to a biphasic pattern, as previously reported [26]. Initial and terminal plasma half-lives were found to be 18.3 min and 81.9 h for total Pt, and 16.9 min and 59.0 h for free Pt, respectively. In the second and third courses investigated both concentrations showed the same trend towards a progressive and statistically significant ($p < 0.025$) increase. This tendency of plasma concentrations to increase for total and free Pt resulted in a concomitant increase in tissue exposure as determined by the area under the concentration *vs* time curve (AUC) (Fig. 4). As the courses proceeded, there was a concurrent progressive decrease in the Pt urinary excretion during the infusion and the following seven days: from 44.1% at the first course, to 36.2% at the second and 28.4% at the third course [27].

As regards the HAP studies, the results obtained evidenced a maximum tolerable Cisplatin dose of 3.2 mg/kg body weight, bolus being more advantageous than fractionated administration. The most suitable temperature level to employ in HAP with Cisplatin seemed to be 41.5 °C [22].

Table 5. - *Toxicity (a) and therapeutic effects (b) observed in 71 courses with Cisplatin and etoposide*

a

Toxicity		Percentage of courses with ECOG grade				
		0	1	2	3	4
Hematologic	- leukopenia	1	13	17	27	42
	- thrombocytopenia	5	10	30	24	31
	- anemia	9	21	4	66	-
Gastrointestinal	- nausea and vomiting	27	24	49	-	-
	- diarrhea	100	-	-	-	-
	- hepatic abnormalities	100	-	-	-	-
Renal	- serum creatinine increase	85	14	1	-	-
Neurologic	- CNS abnormalities (*)	100	-	-	-	-
	- peripheral neuropathy	100	-	-	-	-

b

Response	Percentage of patients	
	Previously treated (n 13)	Previously untreated (n 8)
Complete response	8	-
Partial response	54	100
Minor response	30	-
Stable disease	8	-
Progressive disease	-	-

(*) Other than ototoxicity

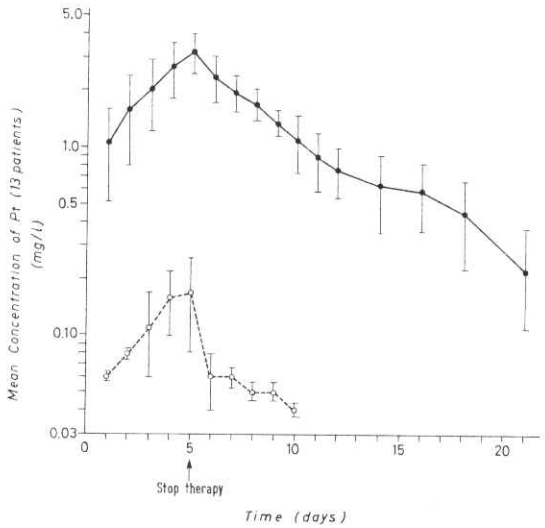

Fig. 3. - Plasma concentrations of total (solid line) and free (broken line) Pt for the first courses and subsequent days.

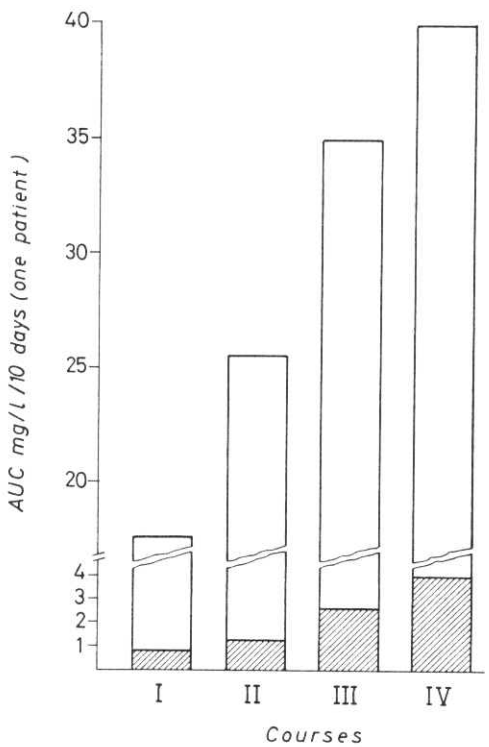

Fig. 4. - Typical AUC values (white area: total Pt; shaded area: so-called free Pt).

Carboplatin

Clinical results and pharmacokinetic evaluation for this agent are in progress. Preliminary evidence, however, substantiates its low toxicity even when administered at high doses (Table 6, part a). Gastrointestinal toxicity and ototoxicity are far below the toxicity observed with high-dose Cisplatin given either by rapid [28] or by continuous [7] infusion. Nephrotoxicity and neurotoxicity never occurred, the only adverse effect being a moderate and still acceptable myelosuppression. In relation to the therapeutic activity, the overall response rate is 7/11 (64%) in pretreated patients and 10/12 (83%) in previously untreated ones (Table 6, part b).

The predictions of a greater bioavailability of Carboplatin compared to Cisplatin, probably due to the higher chemical stability of the first, were confirmed by the experimental findings. In the course of the infusion and immediately following its completion, the percentage of free Pt ranged from 77% to 53% of total Pt, compared to 5% previously demonstrated for Cisplatin. Due to the higher plasma levels of free drug, however, the urinary excretion is even greater and higher doses in shorter periods are thus needed.

Plasma distribution

On the basis of *in vitro* findings, it can be assumed that Pt is bound to albumin and globulin fractions as well as to unidentified protein species of relatively low molecular weight.

In fact, plasma samples incubated with Cisplatin for 2 h at 37 °C, ultrafiltrated with cut-off value at 10,000 Da and then fractionated by HPLC, clearly show that there is a not negligible amount of the element linked to compounds of molecular weight below the mentioned threshold [29]. Thus, these evidences shed further light about the meaning of the term "free Pt", which consists of both bound and actually free Pt species.

Insofar as the binding kinetics of the Pt drugs is concerned, Table 7 lists the various amounts of bound and free Pt at the pre-established incubation times. For Cisplatin the free aliquot is virtually absent 24 h after starting the experiment, while in the case of Carboplatin it is still clearly detectable 120 h later, the half-lives being respectively 81 min (time interval 0-4 h) and 30 h (time interval 0-120 h). This behavior can be traced back to the different nature of ligands present in drug molecule. It can be assumed that the relative ease with which Cl groups are released by Cisplatin makes it more prone to form bonds with receptor groups of proteins. Predictions of a greater availability of free Pt in the case of Carboplatin, as evidenced by *in vitro* experiments, are confirmed by the data obtained in the *in vivo* study.

Foreseeable developments

On the basis of results obtained by our group some conclusions can be drawn regarding the clinical and pharmacological activity of Pt-based drugs as well as the

Table 6. - *Toxicity (a) and therapeutic effects (b) observed in 67 courses with Carboplatin and etoposide*

a

Toxicity		Percentage of courses with ECOG grade				
		0	1	2	3	4
Hematologic	- leukopenia	3	12	33	39	13
	- thrombocytopenia	10	15	39	18	18
	- anemia	24	33	18	25	-
Gastrointestinal	- nausea and vomiting	46	33	21	-	-
	- diarrhea	100	-	-	-	-
	- hepatic abnormalities	100	-	-	-	-
Renal	- serum creatinine increase	100	-	-	-	-
Neurologic	- CNS abnormalities (*)	100	-	-	-	-
	- peripheral neuropathy	100	-	-	-	-

b

Response	Percentage of patients	
	Previously treated (n 11)	Previously untreated (n 12)
Complete response	28	8
Partial response	36	76
Minor response	36	8
Stable disease	-	-
Progressive disease	-	-

(*) Other than ototoxicity

underlying analytical problems. The pharmacokinetic investigation carried out during the Cisplatin treatment confirms that, being nephrotoxicity and ototoxicity associated at maximum Pt plasma level [30, 31], these adverse effects decrease for prolonged infusion schedules. As the courses proceed, the increased tissue exposure to Cisplatin (calculated as AUC) results in an increased myelosuppression, substantiating its dependence on the total exposure to the drug. However, the administration of Cisplatin is always associated with a not negligible toxicity persisting with whatever schedule is adopted. This fact does not allow further significant improvement of its therapeutic index.

Consequently, it becomes mandatory to perform a systematic evaluation of clinical and pharmacological properties of other Pt-based compounds.

As expected from preliminary studies, for Carboplatin the equivalent antitumor activity is combined with a decreased toxicity compared to parent compound. To date, four different schedules of administration have been reported for Carboplatin: a single bolus or 24 hour continuous infusion administration every 4 or 5 weeks; a daily administration for 5 consecutive days every 5 weeks; and, finally, a weekly administration for 4 consecutive weeks every 6 weeks. For each schedule a slightly different phase II dose has been recommended, but all were in the range of 400-500 mg/m^2/course for adults [32]. Further investigations are now being carried out to work out the best therapeutic schedule and to assess Carboplatin's activity on different tumor types.

Moreover, in order to perform systematic monitoring of drug plasma levels it is necessary to plan a sampling protocol which envisages a lower total number of blood specimens. Our group is for the time being working on proving a possible relationship between the overall tissue exposure to the drug (as evaluated by AUC) and Pt level in one and the same representative blood sample. The aim is to standardize chemotherapeutic treatment referring to drug plasma levels instead of conventional dosage related to the body surface area or weight. In addition, the reduc-

Table 7 - In vitro *study: percentage of free and bound drugs*

Incubation times (h)	Cisplatin		Carboplatin	
	Bound Pt (%)	Free Pt (%)	Bound Pt (%)	Free Pt (%)
0	9	91	0	100
2	76	24	20	80
4	90	10	29	71
24	100	0	50	50
48	-	-	78	22
72	-	-	84	16
120	-	-	91	9

tion of blood sampling should decrease the patient's stress and allow a simpler and more straightforward monitoring. In connection with this, the determination of the actual cytotoxic species rather than the "only" ultrafiltrable aliquot, is a further critical step in the monitoring of treated patients. In fact, present analytical findings clearly demonstrate that the ultrafiltrable fraction is a mixture of Pt compounds. Since only the AUC relative to the cytotoxic species might reveal the relationships linking drug tissue exposure to therapeutic and adverse effects, the identification and determination of various ultrafiltrable Pt species becomes obviously mandatory.

Regarding regional Cisplatin perfusion associated with hyperthermia, further studies are needed to confirm these promising preliminary findings and to define the actual therapeutic gain of HAP with Cisplatin compared with other treatments of sarcomas of the limbs.

In an overall evaluation of pharmacokinetics of Pt-based drugs, variations in plasma level of other elements following the treatment cannot be disregarded. In fact, recent studies outlined the possible influence of the drug on the plasma distribution of a number of essential elements [14, 33]. In this framework, new research lines are being launched to evidence the role played by Pt-antitumor agents on plasma disposition of elements such as Cu, Fe, Mg, Se and Zn.

Finally, results available so far substantiate the feasibility of the study utilizing a composite analytical system consisting of an HPLC device and an ICP simultaneous spectrometer for separating plasma fractions and quantitating the different elements in each fraction.

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