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Pharmacokinetics of Cisplatin in Semi-closed Hyperthermic Peritoneal Perfusion (HPP) for Treatment of Peritoneal Carcinomatosis

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Abstract. *Background: This study investigates the pharmacokinetics and toxicity of cisplatin, administered by a new semi-closed hyperthermic peritoneal perfusion (HPP) technique to patients with peritoneal carcinomatosis. Materials and Methods: After surgical cytoreduction, 12 patients were given cisplatin 100 mg/m^2 (CDDP), introduced into the HPP circuit for 60 min at 41.7˚C and 1200 ml/min flow rate. Perfusate and blood samples were obtained during/after perfusion, plus normal and tumor tissues samples before/after perfusion. Results: Total and ultrafiltrate (UF) CDDP had similar patterns: monophasic in peritoneum, biphasic in plasma. At the end of perfusion, total/UF platinum (Pt) concentrations in the peritoneum decreased by 63.4%-64.9%. Total/UF Pt concentrations and AUC_{tot} in perfusate were higher than plasmatic ones. Pt concentrations in tumor specimens were higher than in normal tissues. Conclusion: Cisplatin administered by semi-closed HPP evidenced pharmacological advantages: higher and direct drug exposure of the tumor within the peritoneal cavity, limited systemic absorption and mild toxicity.*

During the past decade, there has been considerable interest in the use of continuous hyperthermic intraperitoneal perfusion (CHPP) for the treatment of peritoneal carcinomatosis (1). Using this technique, immediately after tumor debulking, while the patient is still under anesthesia, a heated chemotherapy solution is circulated through the abdominal cavity at constant flow rate (2). This approach affords high regional delivery of antiblastic drugs, while minimizing systemic toxicity. Cisplatin (CDDP) has shown itself to be a good candidate for intraperitoneal (IP) administration, since it is a large, water-soluble, ionized compound that slowly diffuses from the peritoneal cavity, resulting in a higher peritoneal than plasma concentration (3). *In vitro* studies have shown that, by moderately elevating the temperature, there is an increase of cellular uptake, the extent of DNA cross-linking and the cytotoxic action of cisplatin (4,5). Many pharmacokinetic (pk) analyses of IP CDDP have been reported (6-10); their goal has been to establish a pharmacological advantage of the drug during CHPP treatment by comparing CDDP concentrations in perfusate and plasma. However, there is a considerable spread of results, due to the very different surgical techniques employed and to the different protocols and pk models. Many CHPP techniques have been applied (2): closed, open, with peritoneal expander, *etc.* Different conditions have been: number of implanted catheters, flow rate, intraperitoneal temperature (41-48˚C), perfusion time (from 60 to 120 min). Moreover, the few clinical pk studies in CHPP conditions using cisplatin in monotherapy (7-10) or in association with mitomycin, doxorubicin (11) or TNF (6), usually only analyzed plasma or peritoneal total platinum (total Pt), representing both protein-bound and unbound drug during perfusion. Instead, meaningful pk studies also require determination of the levels of free circulating platinum (UF Pt), which consists of non-protein-bound intact drug and reactive metabolites. These latter species (mainly acquated Pt complex) can react with a variety of nucleophile macromolecules, including plasma proteins, DNA and red blood cells, influencing antitumoral efficacy and many pk parameters (12). One of us (M. De Simone) accomplished 88 interventions between 1995 and 2003 using a new technique: a semi-closed HPP associated with surgical cytoreduction (SC) (13). Our present aims

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were to: 1) validate our semi-closed HPP technique through a pk study of total and UF Pt; 2) determine both total and UF Pt in plasma and peritoneum, thus ascertaining pk differences between plasma and peritoneum; 3) determine whether platinum accumulates in tumor tissues more than in healthy tissues.

Materials and Methods

Patients' characteristics and inclusion criteria. Twelve patients, fulfilling the following criteria, entered the protocol: age between 18 and 70 years, peritoneal carcinomatosis confirmed by cytological or histological examination (six patients had peritoneal carcinomatosis due to peritoneal mixomatosis-*Pseudomixoma peritonei*, one had recurrent retroperitoneal sarcoma, one had malignant mesothelioma, three had advanced metastatic ovarian carcinoma confined to the peritoneal cavity, one had intraperitoneal carcinosis); Karnofsky performance status > 70; absence of extraperitoneal neoplastic illness; absence of intestinal occlusion; absence of significant metabolic diseases of the kidney, liver and cardiorespiratory system; negative pregnancy test for women of fertile age. Informed consent was signed by all patients.

Exclusion criteria. Patient exclusion was decided at the moment of treatment following the direct observation of the abdomen prior to SC. Exclusion criteria were: presence of malignant nodules > 1 cm in size; intrabdominal unresectable metastasis (*i.e.* hepatic or retroperitoneal), creatinine clearance < 50 ml/min.

Treatment plan. The perfusion technique was optimized by M. De Simone, San Giovanni Battista Hospital, Torino, Italy, following a procedure described elsewhere (13). A SC was performed under general anesthesia, before perfusion. Perfusion was performed using a HPP device to heat and pump the peritoneal perfusate into an external sterile closed circuit. Part of the solution usually utilized for peritoneal dialysis was pumped into the circuit and heated to 44˚C. The remainder of the solution was directly heated in an external bath, before the perfusion was poured directly into the abdominal cavity under temperature control (to avoid burning). The liquid volume was calculated taking as reference the entire body surface (2 l/m^2), since the peritoneal surface was considered to be directly proportional to body surface. Perfusion was begun at the end of drug administration (in bolus) and lasted 60 min; the drug mixture consisted of CDDP (100 mg/m^2) associated with mitomycin (16 mg/m^2) or doxorubicin (18 mg/m^2): this latter is usually used in sarcoma or ovarian carcinoma. During HPP, continuous peritoneal temperature was monitored by a series of thermocouples placed into the abdominal cavity: the temperature reached a steady state of 41.7˚C after 30 min. A mean flow-rate of 1200 ml/min was maintained during perfusion. During perfusion, it was also necessary to stir the liquid present in the peritoneal cavity manually. At the end of treatment (60 min), the liquid was completely drained off. During perfusion, blood and perfusate (peritoneal liquid) samples were drawn at specific times; bioptic samples were also collected before and after treatment.

Specimen collection. Blood and perfusate specimens were collected directly in the surgical room. Both blood and circuit specimens were taken before drug administration (basal) and at 5, 10, 20, 30, 45 and 60 min after the start of perfusion. After the end of perfusion, a further two samples were taken, at 90 and 120 min. To evaluate the residual platinum in normal and tumor tissue, a minimum of two samples were taken: immediately before perfusion (basal) and after the end of perfusion. For this purpose, it was necessary to leave at least one unresectable nodule (as tumor tissue specimen) at the end of SC. Specimens were collected up to a maximum of 30 min pre- and post-treatment. Reference normal tissues were liver, peritoneum, muscle and gut; to sample the gut, it was necessary to resect it.

Cisplatin analysis. To collect blood and peritoneal liquid specimens, 6 ml heparinized vials were used. Plasma was obtained after centrifugation at 3500 rpm for 10 min and divided in two parts: 1 ml, used to obtain the ultrafiltrate, was cooled to -20˚C, the remainder of the plasma was cooled to -80˚C. Plasma samples were thawed just before analysis and diluted with HNO_3 for FAAS analysis. Plasma ultrafiltrate was obtained from plasma (1 ml) diluted 1:1 with 0.9% NaCl, using Centricon 10 (Amicon Division, Danvers, USA) following ultracentrifugation (50 min, 5000 rpm). Samples of plasma ultrafiltrate were stored at -20˚C. Immediately before analysis, the plasma ultrafiltrate was diluted with an aqueous solution of HNO_3. A validated method to determine platinum in human plasma and plasma ultrafiltrate was followed (14). A 3030-Z Zeeman spectrometer equipped with a A-60 autosampler (Perkin-Elmer, Norwack, USA) was used. The Pt hollow cathode lamp (Perkin Elmer) operated at a voltage of 35 mV. The samples were deposited in pyrolytically-coated partitioned graphite tubes (Perkin Elmer). Twenty-five ml aliquots of plasma, fortified with 5 µl matrix modifier (Triton X-100 5% water solution), were placed in the pyrolytically-coated tube. Peak heights of absorbance profiles were calculated at 265.9 nm; the instrument automatically calculated the calibration curve by plotting the standards absorbance peak area *vs* time; the regression equation was calculated using minimum squares method.

Pt tissue analysis. Bioptic specimens of normal and tumor tissue were stored at -20˚C. The accumulation of platinum was analyzed by a modified version of the Pera method (15). Briefly, tissue samples were lyophilized in order to relate tissue drug concentration to dry tissue weight. The lyophilized tissue was digested by heating with concentrated HNO_3 and then left to stand overnight in a pyrex tube containing 0.5 ml HNO_3. The tubes were then heated to 90˚C for two hours. After cooling, the clear pale-yellow solution was diluted to volume (5 ml) with milliQ water and stored at -20˚C. All samples were then analyzed directly by FAAS.

Pharmacokinetic analysis. Pk parameters were calculated using Kinetica 4.1.1 (InnaPhase Corp., Philadelphia, USA) following a non-compartmental infusion analysis for perfusate, a non-compartmental absorption analysis and a compartmental zero-order model for plasma. AUC_{tot} and $AUC_{0\text{-}1h}$ were calculated too. The quantity of platinum in the tissue was related to the dry weight of the lyophilized tissue.

Results

Pharmacokinetics. A total of 12 patients who met the eligibility criteria entered this pk study. Figure 1 shows a semi-log plot of total and UF Pt concentrations *versus* time, in plasma and in circuit; the main pk parameters are summarized in Table I. For the plasma, total and UF Pt

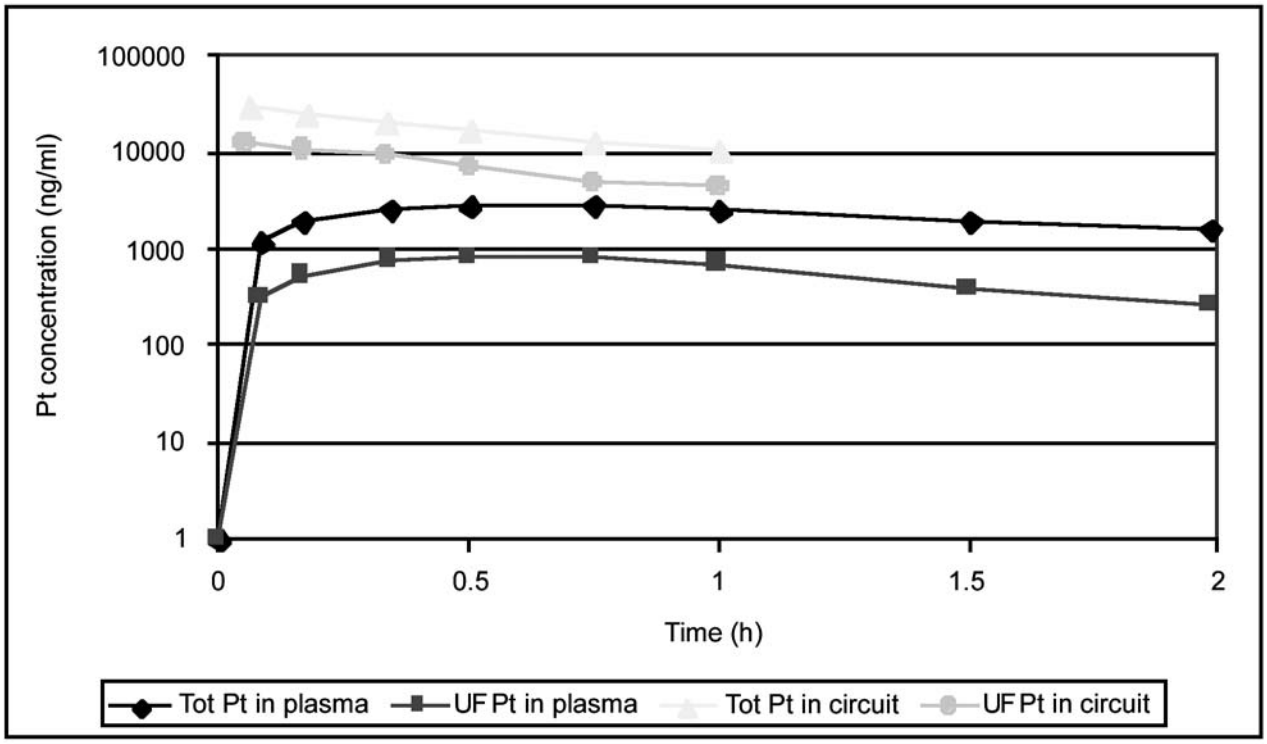

Figure 1. *Total and ultrafiltrate platinum concentrations in plasma and circuit.*

Table I. *Median pharmacokinetic parameters of total and UF Pt in plasma and circuit*.

		Plasma tot Pt	Plasma UF Pt	Circuit tot Pt	Circuit UF Pt
Dose	mg	166	166	166	166
C_{max}	ng/ml	2873.85	855.25	29389.0	13181.1
T_{max}	h	0.63	0.51		
AUC_{tot}	ng*h/ml	7224.01	1227.56	27576.65	10953.57
$AUC_{0\text{-}1h}$	ng*h/ml			18031.95	7738.42
$AUMC_{tot}$	ng*h^2/ml	13246.87	1546.63	29098.43	9765.61
Kel	1/h	0.59	0.97	0.98	1.23
t½	h	1.18	0.72	0.71	0.57
MRT	h	1.95	1.29	0.51	0.37
Cltot	l/h	20.98	102.78	5.40	15.03
V_z	l	37.82	96.53	5.73	13.04
V_{ss}	l	42.89	129.96	3.01	5.43

showed similar biphasic behavior: an absorption phase (0.63 and 0.51 h); median C_{max} 2873.85 and 855.25 ng/ml, followed by a steady state level until the end of perfusion (60 min). One hour after the end of perfusion, the Pt concentration had dropped to 1640 ng/ml (tot Pt; 58.4% of C_{max}) and to 262 ng/ml (UF: 32.5% of C_{max}). The IP total and UF Pt median levels were much higher than in plasma, reaching maximum values of 29389 ng/ml and 13181 ng/ml. In this case, there was a linear monophasic (r^2 0.95) decrease of the median Pt concentrations, reaching minimum levels at the end of perfusion (63.4% and 64.9% decrease for total Pt and UF Pt, in comparison to C_{max} value). The high $AUC_{0\text{-}1h}/AUC_{tot}$ ratio of both Pt species in the peritoneum closely describes the effective drug disposition in peritoneum during perfusion. In the plasma, the high AUC_{tot} total Pt/AUC_{tot} UF Pt ratio (5.88), which

Table II. *Median (range) Pt concentrations in healthy and tumor tissue.*

	Liver	Peritoneum	Muscle	Tumor	Gut
μg Pt/mg dry tissue	0.13 (0.08-0.70)	0.10 (0.03-0.44)	0.17 (0.01-0.35)	0.24 (0.01-0.88)	0.11 (0.09-0.17)

remained constant during perfusion, is related to the known high binding of Pt to plasma proteins or blood cells. Both in perfusate and plasma, the clearance of UF Pt was from three to five times higher than total Pt. In the plasma, this reflects the faster elimination of unbound Pt; in the peritoneum, it was related to the better diffusion of UF Pt through the peritoneal membrane. Further pk analysis of UF Pt concentration in the circuit using a mono-compartmental model revealed an interesting zero order kinetics: thus, the peritoneal membrane acts as a rate-limiting membrane. A definitive pharmacological benefit of cisplatin administered following the semi-closed HPP technique was also observed. On average, the C_{max} values of total and UF Pt in the perfusate were 10.23 and 15.41 times, respectively, higher than those found in the plasma. The same trend was also evident taking the ratio between IP total Pt $AUC_{0\text{-}1h}$/plasma total Pt $AUC_{0\text{-}1h}$ (4.05), or between the IP UF Pt $AUC_{0\text{-}1h}$ and that of plasma (11.14). The favorable cisplatin accumulation in tumor tissue was evaluated by taking a bioptic sample from tumor or healthy tissue, before and after perfusion. A very high variability between the samples was observed (Table II). Nevertheless, in comparison to healthy tissue (liver, peritoneum, muscle and gut), there was a constant higher concentration of the drug in tumor tissue.

Toxicity. A very mild toxicity (grade 1 on the WHO scale) was observed, taking the form of nausea, transient tachycardia, mild neurological disorders, lung toxicity and slight anemia. These symptoms are commonly related to stress due to the length of surgery (10 h). For example, the lung toxicity derived from diaphragmatic peritonectomy, neurological disorders from prolonged anesthesia, nausea caused by morphine and anemia caused by blood loss during surgery. Very common side-effects were also weight loss and asthenia, post-operative fever and diarrhea (due to infection). The examination of treatment toxicity 8 days after surgery gave the following results: six patients showed no post-operative toxicity, two underwent ileus perforation, two ascites (one mild, one massive), one developed urethral fistula (resolved in two days), one sub-occlusion episode (spontaneously resolved). No cisplatin toxicity (nephrotoxicity, neurotoxicity or bone marrow suppression) was observed.

Discussion

Peritoneal carcinomatosis is currently considered untreatable, with few exceptions, and is associated with a dismal prognosis (16). CHPP associated with prior surgical debulking (SC) is considered a new aggressive strategy to treat peritoneal carcinomatosis. Cisplatin is considered to be one of the most effective drugs used in IP and hyperthermal therapy of peritoneal carcinomatosis. However, few studies have reported the use of cisplatin with the CHPP associated to SC; none have used semi-closed HPP, as applied here. In this study, we found a pharmacological advantage to HPP administration of cisplatin, taking into account different parameters: the ratio of median C_{max} of total/UF Pt in perfusate and plasma and the ratio between IP tot Pt $AUC_{0\text{-}1h}$ and IP UF Pt $AUC_{0\text{-}1h}$. Low total and UF plasma cisplatin levels accounted for the very mild toxicity observed. Through the pk study, we also validated the drug protocol (dosage, perfusion time) together with the semi-closed HPP technique. The pharmacological efficiency was also seen by the observation that, in the peritoneum, the level of tot Pt was constantly above that considered the efficacy level (6 μg/ml) (4). A monophasic pattern of both total and UF Pt levels in the circuit during perfusion gave a clear indication that there was no saturation phase during perfusion and that t½ remained fairly constant. It has been shown that a linear relationship of UF Pt concentration/time is maintained until 60 min (17), which was our perfusion time. It has been demonstrated, both *in vitro* and *in vivo,* that heating or increased infusion time accelerates the formation of active free Pt metabolites, causing increased levels of bound Pt in plasma over time (5,18). On heating, more IP UF Pt was formed; this latter, instead of binding to blood cells, as in plasma, was strongly taken up by tumor cells. This was shown by the higher median Pt level in IP tumor nodules than in healthy tissues. Heating also increased the permeability through the peritoneum membrane, giving a higher clearance of UF Pt (cytotoxic Pt) in comparison to total Pt. However, the two opposite phenomena were well balanced, since the IP UF Pt level over 1-h perfusion at 41.7˚C remained for most of the time above that considered clinically effective, confirming that the peritoneum membrane still acted as a semi-permeable barrier, favoring

accumulation of cisplatin. In conclusion, IP administration of cisplatin, by combining hyperthermal treatment and SC using a new technique, appeared particularly advantageous in terms of efficacy, toxicity and pk behavior. These results provide a rationale for further exploration of this combined HPP plus SC treatment with cisplatin, given the mild toxicity observed.

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