Serum and intraperitoneal pharmacokinetics of cisplatin within intraoperative intraperitoneal chemotherapy: influence of protein binding

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Intraperitoneal (i.p.) chemotherapy is a promising therapeutic method to improve the effectiveness of cisplatin in patients with ovarian cancer and peritoneum involvement. Intraperitoneal treatment can be intraoperatively performed just after a complete surgical resection of peritoneal tumor nodules. However, little is known regarding the pharmacokinetics of platinum during intraoperative i.p. chemotherapy (IIC). Serum and i.p. measurements of total and ultrafilterable platinum were performed to determined pharmacokinetic parameters in 11 consecutive patients who received a 2-h IIC with 50 mg/m^2 cisplatin. Protein concentrations were determined in serum and peritoneal liquid at the same times. The cisplatin concentration required to kill OVCAR-3 human ovarian cancer cells and evaluation of cisplatin binding to proteins were determined *in vitro*. Platinum i.p. concentration decreased rapidly and quickly came under the cytotoxicity threshold (10 mg for 2 h). About 85% of i.p. and serum cisplatin was ultrafilterable during IIC. Platinum concentrations were closely related to protein concentrations. Due to the very low level of serum protein (almost 25 g/l), serum cisplatin binding during chemotherapy was very low (almost 25%), but increased with protein concentration recovery. These pharmacokinetic data show that a sufficient concentration to kill human ovarian cancer is not reached with a single i.p. bath containing 50 mg/m^2 cisplatin for 2 h. A new protocol with a renewed bath and a higher cisplatin concentration is under investigation. *Anti-Cancer Drugs* 16:1009–1016

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Introduction

Ovarian cancer is the main gynecologic cause of death in the western world and the fifth cause of cancer death among women [1–3]. Such mortality is partly due to a delayed diagnosis which is commonly established when peritoneum and/or visceral metastasis involvement occurs. Standard treatments including exploratory laparotomy with cytoreductive surgery and chemotherapy based on platinum/taxanes combinations are proposed [4].

The rationale for intraperitoneal (i.p.) chemotherapy for patients with ovarian cancer and peritoneum involvement is high drug concentration exposure leading to an increased cytotoxicity and avoiding a high level of systemic toxicity. Preliminary results using i.p. cisplatin were encouraging and provided 30–56% objective response rates [5–7]. Comparison between i.p. and i.v. treatment was undertaken in several studies. Results stressed the low level of toxicity [8,9] with favorable trends in terms of overall and/or progression-free survival for treatment including i.p. chemotherapy [3,10,11]. Intraperitoneal chemotherapy is an encouraging treatment option for ovarian cancer with peritoneum involvement in combination with i.v. chemotherapy [12,13].

The most commonly used drug for i.p. treatment is cisplatin. Cisplatin is a heavy metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the *cis* position. Its pharmacodynamic and pharmacokinetic properties support its use for i.p. administration. Comparison of i.p. and i.v. cisplatin treatments showed that the ratios in terms of exposure or peak concentrations are 12 and 20, respectively [12,13]. The relationship between concentration/exposure and efficacy is well established, and supports the interest for local administration [13,14].

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The dose-limiting toxicity seems to be systemic rather than local [13].

Intraperitoneal administration is commonly performed at a distance from surgery by an i.p. catheter with artificial ascites [12,13]. A therapeutic proposal is to treat during surgery with the aim of enhancing the diffusion of cisplatin, which should be optimal [15]. The present study reports the first pharmacokinetic analysis of open i.p. cisplatin treatment performed during surgery.

Patients and methods

Cancer cells and cytotoxicity assay

The OVCAR-3 human ovarian cancer cell line was obtained from the ATCC (Manassas, Virginia; USA; HTB-161). This cell line is a good model for resistance studies since it was established from the malignant ascites of a patient with a progressive ovary adenocarcinoma despite treatment with doxorubicin, melphalan and cisplatin [16]. Cells were cultivated in RPMI 1640 medium supplemented with 10% FBS. Cells were detached before experimentation with a mixture of 1 g/l trypsin and 0.4 g/l EDTA in HBSS. A modified clonogenic assay was used to assess cell survival after cisplatin. Cells (10^5/well) were seeded in 1 ml of culture medium in 24-well tissue culture plates and cultivated for 96 h until confluent. After 2 h incubation with cisplatin at various concentrations, wells were rinsed twice. Cells were trypsinized for 15 min and then suspended in culture medium with FBS to inhibit trypsin. Aliquots of 10^4 cells/well in 200 µl culture medium were seeded in 96-well culture plates. Cells were cultivated for 10 days in drug-free culture medium, with renewal of the culture medium at day 5. Cytotoxicity was measured by a colorimetric crystal violet assay. Wells were rinsed with PBS, then fixed with pure ethanol and dried. Cells were stained for 5 min with crystal violet (1 g/ml in distilled water). Excess dye was flushed with water and cell-bound dye was eluted with 33% acetic acid. Plates were read at a wavelength of 540 nm on an automatic spectrophotometer.

Patients and treatment

Between November 2003 and July 2004, 11 patients with advanced epithelial ovarian cancer classified FIGO stage IIIC were included. Median age at the time of diagnosis was 59.5 years (range 44.5–75.7). All patients were PS 0 or 1. Tumors were undifferentiated carcinoma, poorly and moderately differentiated in two, three and six cases, respectively. Cell types were serous (six of 11), mucinous (two of 11), undifferentiated (two of 11) and endometrioid (one of 11).

In five patients, treatment included 4 cycles of i.v. induction chemotherapy with paclitaxel and carboplatin followed by a debulking surgery with cisplatin i.p. treatment. Then, an additional 4 cycles of paclitaxel/carboplatin chemotherapy were administered. In six patients, treatment included an initial debulking surgery followed by 6 cycles of i.v. paclitaxel/carboplatin chemotherapy. Second-look surgery with i.p. cisplatin chemotherapy was then performed.

After the optimal cytoreductive surgery was achieved in all patients, intraoperative i.p. chemotherapy (IIC) was performed during surgery by filling the peritoneal cavity with 3 l of isotonic saline pre-heated at 37°C and a total dose of 50 mg/m^2 cisplatin for 2 h. Optimal diffusion was obtained by stirring by hand for the 2 h. Then the peritoneal cavity was cleared out and rinsed before closing down. Concomitant i.v. hydration with 3000 ml normal saline, 2.2 mM Ca^{2+} glucuronate, 1 g/l Mg^{2+}, 2 g/l KCl and 3 g/l NaCl was administered to prevent renal toxicity.

This study was conducted in accordance with the principles embodied in the Declaration of Helsinki.

Pharmacokinetic study

Peritoneal samples were collected at 5, 30, 60, 90 and 120 min after the beginning of cisplatin administration; blood samples were drawn at 30, 60 and 120 min after the beginning of cisplatin administration. After the completion of IIC, additional blood samples were collected at 12, 16 and 24 h. Blood samples were centrifuged at 4°C. Separation of ultrafilterable platinum from serum and peritoneal liquid was performed by ultrafiltration at 4°C (Amicon Ultra-4 centrifugal filter devices; Millipore, Bedford, Massachusetts, USA). No platinum adsorption to the filtration device was detected. All samples were frozen at −20°C until assay.

Platinum concentration measurements were performed by inductively-coupled plasma mass spectrometry (ICP-MS). A Hewlett Packard (Les Ulis, France) model 4500 ICP-MS was used. The flow of argon gas was delivered with an outer gas flow rate of 15 l/min and a nebulizer (cross-flow) flow rate of 1 l/min. The MS was equipped with a two-cone interface and a quadrupole mass analyzer. The sample uptake rate was 0.2 ml/min. The ions selected from platinum and bismuth (internal standard) were measured at m/z values of 195 and 209, respectively. Platinum standard solution, bismuth solution and nitric acid were purchased from Merck (Darmstadt, Germany). All chemicals and reagents were of the highest available grade, and were used without further purification. The samples were prepared by diluting 50 µl serum with 4950 µl diluent containing 1% nitric acid and 10 µg/l bismuth (around 100-fold dilutions). Calibration curves for platinum were prepared in serum obtained from healthy volunteers and spiked with working standard solution to cover the concentration range of 0–1000 µg/l.

The standards were diluted 100-fold with the same diluent as the samples before being introduced into the nebulization chamber. The limit of detection, calculated as being equivalent to the mean blank value plus 3 SD, was 0.01 µg/l. The lower limit of quantification, defined as 10 SD above the mean blank value, was 0.2 µg/l. The calibration curves exhibited good linearity over the working concentration range of 0–1000 µg/l. Recovery of platinum added to serum was close to 100% at concentrations of 20 and 200 µg/l. Within-run and between-run coefficients of variation were less than 5% for both concentrations studied.

The total or ultrafiltered (Uf) platinum concentrations were fitted separately using PKAnalyst pharmacokinetic software (MicroMath Research, Salt Lake City, Utah, USA). The peritoneal liquid platinum parameters were fitted using a one-compartment model [17] and the blood platinum parameters with a two-compartment model [18–21]. For each patient, the area under the serum concentration–time curve (AUC) for 2 h (peritoneal liquid) or infinity (serum), the maximal concentration (C_{max}), the time to reach maximal concentration (t_{max}), the half-life of overall elimination ($t_{1/2}$), the clearance, and the elimination rate constant (k_e) were determined for total and Uf platinum in serum and peritoneal liquid. Peritoneal clearance was obtained by dividing infinite AUC by the dose of cisplatin administered.

For each sample, the protein concentration was determined by an automated Biuret method (Dade Behring, Paris La Défense, France). Specific albumin concentration was measured in i.p. liquid at 5, 60 and 120 min of the IIC using an IMMAGE system (Beckman Coulter, Roissy, France).

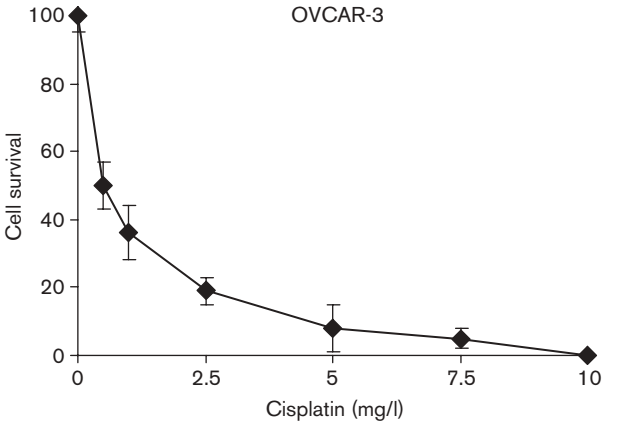
Binding of cisplatin to proteins

Two types of experiments were conducted to study the effect of protein concentration on percentage of binding and the effect of cisplatin concentration on its own binding. In a first experiment, cisplatin at different concentrations (0.1, 0.5, 1 and 5 mg/l) was incubated at 37°C under gentle stirring in human serum whose protein concentration was set at 30 g/l. In a second experiment, cisplatin at 1 mg/l was incubated in serum with various protein concentrations of 20, 30, 45 and 60 g/l. In both experiments, sampling was performed at 15, 60, 120, 240 and 480 min, and partly ultracentrifuged in order to calculate the Uf and total platinum.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis using parametric tests (ANOVA) was performed for variables with a normal distribution. All variables were analyzed using Statview software (SAS Institute, Cary, North Carolina, USA).

Fig. 1



Cytotoxicity of cisplatin on human ovarian cancer cells. Confluent OVCAR-3 cells were treated for 2 h with cisplatin in isotonic saline. Cell survival was assessed by a clonogenic colorimetric assay and presented as the percentage of untreated control cells. Each point is the mean ± SD of four wells of two independent experiments.

Results

Determination of *in vitro* cytotoxic threshold

In order to determine the minimal concentration and time for complete cytotoxicity of cisplatin on relatively resistant cells, OVCAR-3 were treated for 2 h and cell survival was assessed by a modified clonogenic assay (Fig. 1). Complete inhibition of cell growth was obtained for 10 mg/l cisplatin. This threshold concentration should be ideally maintained for 2 h in the peritoneal cavity of patients with ovarian cancer resistant to i.v. cisplatin.

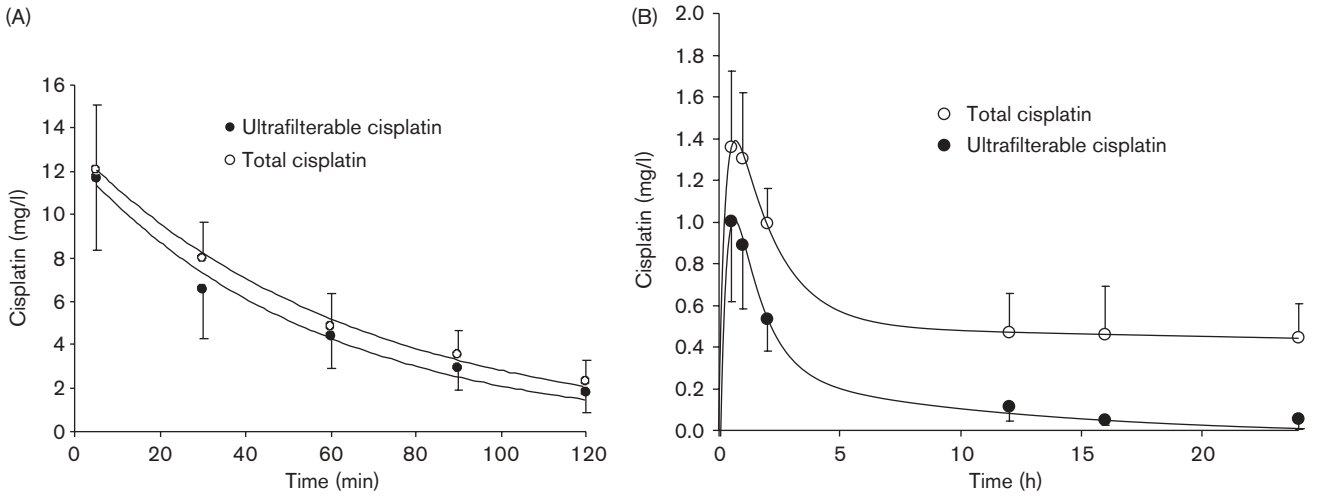
Pharmacokinetic parameters in peritoneal fluid

Figure 2(A) illustrates the mean concentrations of Uf and total platinum in peritoneal liquid during IIC. Intraperitoneal pharmacokinetic parameters of platinum are summarized in Table 1. The concentration of Uf platinum decreased under the targeted 10 mg/l threshold only 15 min after starting the IIC. No statistical difference was observed in the percentages of platinum binding throughout the length of treatment (3.11 ± 13.14, 18.84 ± 18.05, 7.81 ± 21.65, 16.15 ± 16.61 and 20.32 ± 31.00% at 5, 30, 60, 90 and 120 min, respectively). The total protein concentrations increased during the IIC (Fig. 3) and the concentrations almost trebled during this treatment. This increase could be mainly put down to albumin as its concentrations were 0.95 ± 0.55, 2.89 ± 0.90 and 4.46 ± 1.40 mg/l after 5, 60 and 120 min of IIC, respectively. The values of the $AUC_{0-2\,h}$ and k_e of Uf and total i.p. platinum were very close. The ratio between $AUC_{0-2\,h}$ serum/$AUC_{0-2\,h}$ i.p. of Uf platinum was 2.88 ± 1.57.

Pharmacokinetic parameters in serum

The evolution through time of the mean Uf and total platinum concentrations in patient's serum is illustrated

Fig. 2



Intraperitoneal (A) and serum (B) platinum concentrations during and after IIC with 50 mg/m^2 cisplatin in 3 l of isotonic saline. Results are expressed as mean ± SD of 11 patients.

Table 1 Intraperitoneal and serum pharmacokinetic parameters of Uf and total platinum after i.p. administration

	$t_{1/2}$ of elimination (h)	AUC[a] (mg·h/l)	k_e of elimination (h^{-1})	C_{max} (mg/l)	t_{max} (min)	Peritoneal clearance (l/h)
Peritoneal liquid						
total platinum	0.91 ± 0.62	11.73 ± 2.44	0.95 ± 0.38			
Uf platinum	0.73 ± 0.33	10.23 ± 2.91	1.09 ± 0.40			7.14 ± 2.41
Serum						
total platinum	12.03 ± 4.96	46.26 ± 35.30	0.066 ± 0.023			
Uf platinum	1.71 ± 0.52	4.02 ± 1.22	0.46 ± 0.23	1.35 ± 0.85	36.15 ± 8.84	

Samples of peritoneal liquid were collected in 11 patients who received 50 mg/m^2 cisplatin in 3 l of isotonic saline for 2 h. Values are expressed as mean ± SD.
[a]AUCs were calculated for 2 h (i.p.) or infinity (serum).

in Fig. 2(B) and the pharmacokinetic parameters of platinum in serum are summarized in Table 1. A noteworthy difference between Uf and total platinum parameters was observed for delayed samples. This is expressed by a statistically significantly different value of the k_e (0.46 ± 0.23 versus 0.066 ± 0.023 h^{-1} for Uf and total platinum concentration; $P < 0.01$).

Platinum binding was low during the i.p. treatment. Indeed, the mean percentages of platinum binding were 26.39 ± 14.38, 33.56 ± 16.41, 46.63 ± 10.82, 74.94 ± 12.40, 86.06 ± 7.39 and 84.54 ± 14.67%, respectively, at 5, 60 and 120 min, and 12, 16 and 24 h after the beginning of the IIC.

The concentration of protein in serum through time was very low during the IIC with mean values of 26.25 ± 5.33, 25.76 ± 5.93 and 27.13 ± 6.02 g/l at 30, 60 and 120 min after the beginning of the IIC. The concentrations increased after surgery, reaching mean values of 37.40 ± 4.72, 38.20 ± 6.83 and 41.00 ± 4.34 g/l 12, 16 and 24 h.

To assess a potential interaction between protein concentration and platinum binding, a correlation analysis between Uf i.p. and serum protein concentrations was performed (Fig. 4). Concentrations of Uf platinum in serum were inversely correlated to protein concentrations (slope = −0.04, $r^2 = 0.52$, $P < 0.001$).

Binding exploration

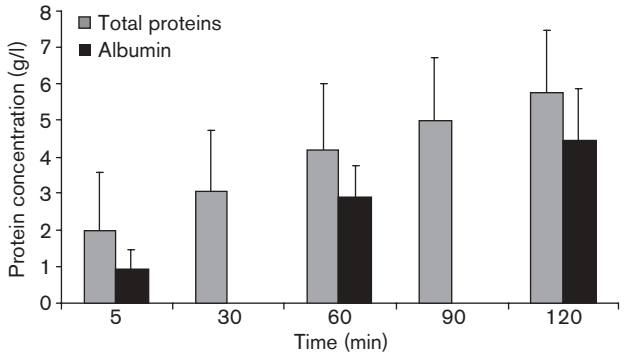
The previous clinical results suggested a low binding of platinum when the protein level is low. This issue was investigated by *in vitro* experiments. In the first set of experiments (Fig. 5A), a clear relation between protein concentration, time and binding was observed. A low percentage of binding appeared to be linked to a low protein level. This link was not related to the platinum level. The binding of platinum at different concentrations and at a fixed protein concentration was only related to the time of incubation (Fig. 5B).

Discussion

This present study was conducted with the goal to optimize IIC. This is the first i.p. pharmacokinetic analysis taking into the account the cytotoxic effect of platinum and impact of platinum binding to proteins. We observed that platinum concentrations quickly decreased

in peritoneal liquid. About 15 min after starting the IIC, the platinum concentration was under the cytotoxicity threshold which had been defined *in vitro*. However, the concentration of platinum in peritoneal liquid remained above 5 mg/l for more than 50 min and above 2.5 mg/l for more than 90 min. This maintained a concentration which killed more than 90% (5 mg/l) of OVCAR cells (considered as a resistant cell line) for 50 min and which killed more than 80% of these cells for 90 min.

Fig. 3



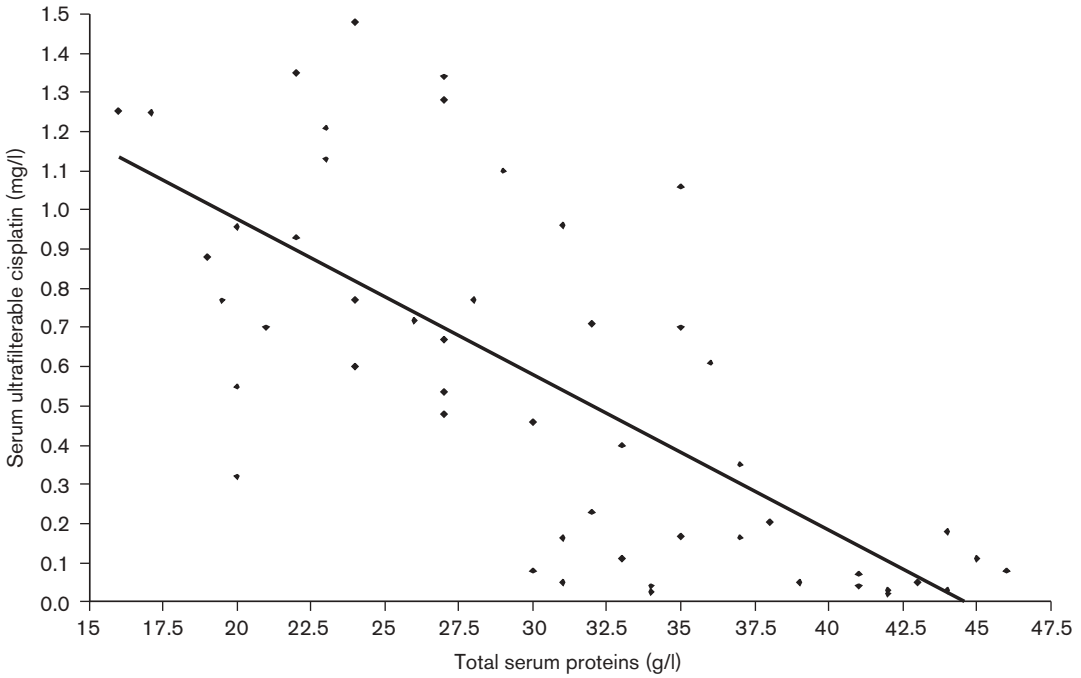
Intraperitoneal protein concentrations during a 2-h IIC with 50 mg/m^2 cisplatin. Results are expressed as mean ± SD of 11 patients.

The rate of platinum binding to the protein in the peritoneal liquid remained stable and low through time even if the concentration of protein increased. This increase, mainly due to albumin, was not sufficient to modify the binding percentage as the capacity of binding was probably exceeded by the high concentration of i.p. platinum (10 mg/l). As a consequence, the $AUC_{0-2\,h}$ and k_e parameters were similar for Uf and total platinum. Thus, more than 85% of the administered cisplatin was available for anti-tumor activity, but also for toxicity and bloodstream transfer. Taking into account the difference in administered doses, the results were of the same order as those previously reported for $t_{1/2}$ or AUCs (Table 2).

The following points were drawn from serum pharmacokinetic parameter analysis. Binding of platinum with serum protein within IIC is lower than that observed following i.v. administration. Binding rates obtained in the present study ranged between 26.39 and 46.63 versus 85–90% reported in studies using i.v. perfusion [22–26].

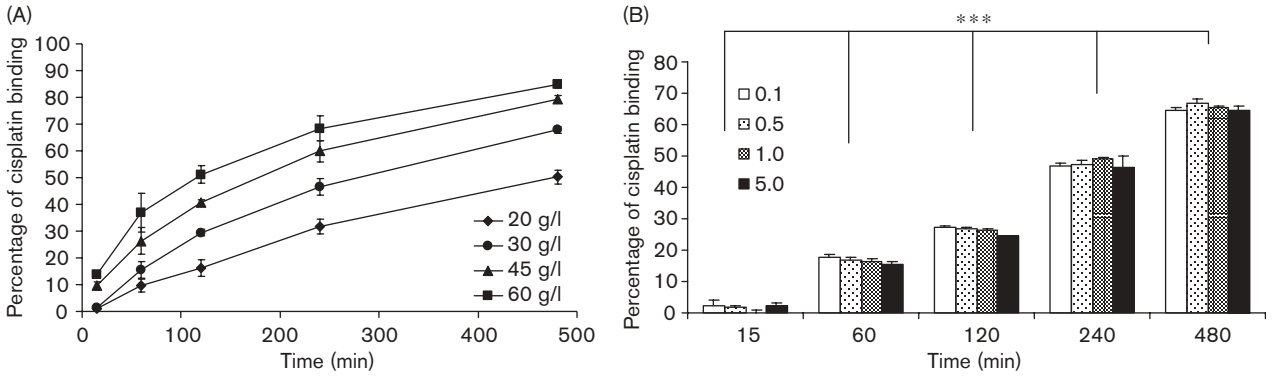
The low protein serum concentration observed during surgery for ovarian cancer could explain these results. Platinum pharmacokinetic studies with i.v. perfusion reported 70 g/l as the mean serum protein concentration in contrast to 27 g/l observed in the present study [18,23,24,27,28]. Such an unusually low level of protein concentration might be explained by both the severity of

Fig. 4



Correlation between serum protein concentration and Uf platinum concentration: slope = 2.41 ($P < 0.001$), squared correlation coefficient = 0.55.

Fig. 5



Percentage of platinum t binding to human serum proteins with time and protein concentration. In the first set, human serum adjusted to 20, 30, 45 and 60 g/l of protein was incubated at 37°C with 1 mg/l platinum (A). In the second set, human serum adjusted to 30 g/l of protein with normal saline was incubated with 0.1, 0.5, 1 and 5 mg/l platinum (B). Results are expressed as mean ± SD of three *in vitro* experiments. ***$P<0.001$ when comparing the different times using a parametric ANOVA.

Table 2 Comparison of the results with data from the literature regarding $t_{1/2}$ and AUC of platinum

	i.p. administration				i.v. administration	
	i.p. parameters	References	Serum parameters	References	Parameters	References
$t_{1/2}$ (h^{-1})	0.48 ± 0.10	[17]				
	1.66	[20]				
	0.73 ± 0.33	present study				
AUC of Uf platinum (mg·h/l)	29.27 ± 9.08 (150 mg/m^2)	[17]	9.51 ± 0.87 (150 mg/m^2)	[17]	11.62 ± 3.37 (150 mg/m^2)	[17,29]
	108.9 ± 55.7 (extrapolated to infinity)	[20]	from 1.44 to 2.19 (50 and 70 mg/m^2)	[30]	3.3 ± 1.5 (100 mg/m^2)	[31]
	10.23 ± 2.91 (50 mg/m^2)	present study	5.77 ± 6.48 (200 mg/m^2)	[20]	2.58 ± 1.62 (150 mg/m^2)	[25]
			4.02 ± 1.22 (50 mg/m^2)	present study	2.34 ± 1.52 (70 mg/m^2)	[32]
					5.50 ± 0.90 (100 mg/m^2)	[26]
					0.55 (100 mg/m^2)	[27]
					0.61 (80 mg/m^2)	[23]
					4.02 ± 1.22 (50 mg/m^2)	present study
AUC of total platinum (mg·h/l)	75.3 ± 29.2 (300 mg/m^2)	[33]				
	10.23 ± 2.91 (50 mg/m^2)	present study				

surgery and administration of high amounts of plasma substitutes. The surgery also implies an omentectomy, and many resections of macro and microscopic disseminations, as well as an exhaustive look at the peritoneal cavity. The surgeries lasted for 7–9 h and up to 13 h for one of the patients in the present study. Interestingly, modification of kinetic parameters for platinum by serum protein concentration was also sometimes observed during i.v. perfusion [18,28]. In the present study, a relationship was found between low protein level and low Uf platinum concentration (Fig. 4), illustrated by an *in vitro* experiment which clearly illustrates that the binding rate of platinum was related to serum protein concentration and time (Fig. 5A). It should be noticed here that the protein level we encountered during surgery is exceptionally encountered during clinical studies. This situation allowed us to perform an analysis of platinum binding under extreme conditions, thus allowing a better understanding of the platinum binding process.

This low binding rate explained why the AUC of Uf platinum obtained after IIC was similar to cisplatin administered by i.v. perfusion at 100 mg/m^2 (Table 2). Such results should not lead to misinterpretation regarding the administered doses. The serum Uf platinum level is high during IIC due to the low protein concentration and to the low binding rate (Table 2). In contrast, the level of serum Uf platinum is lower after i.v. administration [22–26]. Considering such differences, one can conclude that i.p. administration of cisplatin at 50 mg/m^2 during surgery for ovarian cancer leads to a similar exposure as i.v. administration of cisplatin at 100 mg/m^2.

Comparing the serum AUC of Uf platinum obtained in this study with pre-operative administration and those values reported in other types of IIC, it seems that the exposures were similar (Table 2). Also, the ratio between i.p. and serum platinum concentrations is at the lower end of the ranges previously described [12,14,20].

In studies of i.p. chemotherapy performed by Kern *et al.* [17] and Panteix *et al.* [21], the reported values for $t_{1/2}$ were 93 ± 73 h (for Uf platinum) and 79.7 h (for total platinum), respectively. In the present study with IIC the $t_{1/2}$ was 1.71 ± 0.52 h, and an increase of the protein concentration was observed after the IIC (Fig. 3). This increase was associated with an increase of the binding of platinum. This results in a decrease of $t_{1/2}$ and an increase of k_e for Uf platinum. This point is striking in Fig. 2(B): the slope of the final elimination (and the associated k_e) of Uf platinum was higher than that for total platinum.

With the aim of optimizing the IIC, two approaches may be considered – enhancing the concentration of cisplatin administered during the IIC and limiting the systemic spread. An increase of cisplatin posology would prolong the length of time with the platinum concentration above the cytotoxicity level. However, such an increase would induce a higher systemic peak which may induce a dose-limiting toxicity. With this aim of prolonging the efficient length of time, dividing the administration of cisplatin may be considered as an interesting option. Perfusion of albumin might also be discussed during such surgery for ovarian cancer with the aim to correct the low level of serum protein and to avoid a high level of systemic exposure to Uf platinum.

To conclude, IIC with cisplatin associated to exhaustive surgery is under investigation for patients with ovarian cancer and residual peritoneum involvement after i.v. chemotherapy. A single administration of 50 mg/m^2 cisplatin for 2-h IIC resulted in a rapid decrease below the desired threshold, but led to a similar exposure as a 100 mg/m^2 i.v. protocol. As the toxicity was nevertheless low at a dose of 50 mg/m^2, we are presently investigating the pharmacokinetics and tolerance of the same dose divided in two administrations with the goal to increase the length of tumor exposure at a local cytotoxic dose.

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