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# A pharmacogically guided phase I study of carboplatin in combination with methotrexate and vinblastine in advanced urothelial cancer

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**Abstract** Carboplatin is an alternative for cisplatin in the treatment of urothelial cancers. A pharmacologically guided phase I study of carboplatin in combination with methotrexate (30 mg/m$^2$) and vinblastine (4 mg/m$^2$) was conducted in ten patients by increment of the area under the plasma concentration versus time curve (AUC) for ultrafilterable carboplatin using the Calvert formula. The maximal tolerated AUC was 5 mg ml$^{-1}$ min, with neutropenia being the dose-limiting toxicity. There was a significant linear correlation between the percentage of decrease in neutrophil count and the carboplatin AUC. Determination of the glomerular filtration rate by the isotopic method allowed us to adapt the dose of carboplatin given to patients suffering from urothelial cancer, who frequently have impaired renal function. The recommended AUC for phase II study is 4 mg ml$^{-1}$ min.

**Key words** Carboplatin · Urothelial cancer · Phase I trial

## Introduction

Combination therapy with cisplatin, methotrexate, and vinblastine (CMV) represents one of the standard treatments for urothelial cancer [9]. The use of cisplatin in most patients is limited by reduced renal function. Moreover, cisplatin-induced toxic effects represent an important limitation for application of the protocol in elderly patients. Carboplatin has been given to these patients without inducing further impairment: the carboplatin dose ranged between 300 [1,11] and 400 mg/m$^2$ [14] but was reduced according to different parameters, i.e., either measured creatinine clearance [1, 11] or glomerular filtration rate (GFR) and age [14]. The combination of carboplatin, methotrexate (30 mg/m$^2$), and vinblastine (3 mg/m$^2$; Carbo-MV) has yielded an overall response rate of 48% in patients with bladder cancer who are not eligible for cisplatin therapy [1].

Nearly 70% of the delivered carboplatin dose is excreted unchanged in the urine [19, 20]. Renal impairment, especially frequent in patients with urothelial cancer, leads to slower excretion and a higher area under the plasma concentration versus time curve (AUC) for ultrafilterable carboplatin. Moreover, close relationships between this AUC and the thrombocytopenia induced by carboplatin given as a single agent have been shown [2, 5, 6]. In monochemotherapy regimens, target AUC values of 5 and 7 mg ml$^{-1}$ min are recommended for previously treated and untreated patients, respectively, to obtain a manageable degree of hematological toxicity. Since hematological toxicity is increased when carboplatin is given in association with other cytotoxic drugs, the target AUC must be determined for each protocol.

We designed a pharmacologically guided phase I study based on an increment of the AUC to determine the target AUC for carboplatin given in the Carbo-MV combination to patients suffering from urothelial cancer. All of the patients had impaired renal function.

## Patients and methods

### Eligibility criteria

Eligible patients had to be suffering from locally advanced and/or metastatic histologically proven transitional-cell carcinoma; to be 75 years old or younger; to have a performance status (WHO) of 2 or less; to have received no previous treatment; to have impaired renal function [GFR as determined by [$^{51}$Cr]-ethylenediaminetetracetic acid (EDTA) clearance measurement, < 100 ml/min, and/or serum

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creatinine level (Scr), > 120 μmol/l]; and to have a neutrophil count of > 1,500/mm$^3$ and a platelet count of > 150,000/mm$^3$. The protocol was approved by the regional ethics committee. Informed consent was obtained from the patients.

### Experimental design and treatment plan

The carboplatin dose was calculated according to the Calvert formula [2]: dose (mg) = AUC × (GFR + 25). The GFR was determined by [$^{51}$Cr]-EDTA clearance measurement [3] and was expressed as the absolute value (in milliliters per minute) without correction for the body surface area. The starting AUC was 3 mg ml$^{-1}$ min and was increased in increments of 1 mg ml$^{-1}$ min (AUC level: 3, 4, and 5 mg ml$^{-1}$ min). Three patients were to be treated at each AUC level prior to escalation. The AUC level was increased within and between patients.

The chemotherapy was given in 21-day cycles as follows. Methotrexate was given by i.v. infusion at 30 mg/m$^2$ in 20 ml distilled water, and vinblastine was given at 4 mg/m$^2$ into the line of this infusion; the two drugs were given on day 1 and day 8. On day 2, carboplatin was given as a 1-h continuous infusion in 500 ml 5% dextrose. Cycles were delayed for 1 week at day 8 or day 21 if the neutrophil count was < 1,500/mm$^3$ and/or the platelet count was < 100,000/mm$^3$.

### Sample collection

Blood samples (4 ml) were collected from an indwelling i.v. cannula placed in the arm opposite to that receiving the drugs. Samples were taken into heparinized tubes prior to the start of carboplatin administration, at the end of the infusion, and at 0.5, 1, 4, 8, and 24 h after the end of the infusion. After immediate centrifugation at 4°C at 1,500 *g* for 10 min, the plasma was separated and ultrafiltered through an Amicon Centrifee MPS1 system with YMT membranes at 4°C for 30 min at 2,000 *g*. The ultrafiltered plasma was frozen at − 20°C until carboplatin analysis. A part of the plasma sampled prior to the infusion and at 24 h after the infusion was also kept at − 20°C for methotrexate analysis.

### Drug analyses

Plasma ultrafilterable carboplatin levels were measured by flameless atomic absorption spectrophotometric analysis of the platinum as previously described [12]. All results are expressed in micrograms of carboplatin per milliliter, not as elemental platinum. Plasma methotrexate levels were determined by an enzymatic method, i.e., inhibition of the dihydrofolate reductase activity on a Cobas-Bio analyzer (Roche) [10].

### Pharmacokinetic analysis

The ultrafilterable plasma concentrations of carboplatin were fitted using the SIPHAR computer program (Simed, Créteil, France) according to a two-compartment model with the weighting factor 1/ycalc. The AUC (from zero to infinity) was obtained using the fitted model.

### Toxicity evaluation

Hematological parameters were evaluated at weekly intervals, and a physical examination and a serum chemistry survey were performed at each cycle. Linear regression analysis and sigmoidal models ($E = Emax \times AUC/EC50 + AUC$ and $E = Emax \times AUC^H/EC50^H + AUC^H$) were performed using the MICROPHARM computer program (S Urien, Créteil, France) to fit the relationships between the carboplatin AUC and the percentages of reduction in absolute neutrophil and platelet counts.

### Response criteria

A complete response was defined as the complete disappearance of all clinically detectable soft-tissue and visceral malignant disease for at least 4 weeks as determined by physical examination and radiography studies. A partial response was defined as a decrease of 50% or more in the sum of the products of the two longer perpendicular diameters of measurable lesions that lasted for at least 4 weeks without an increase occurring in any site known to contain malignant disease. Progressive disease was defined as an increase of at least 25% in the size of measurable lesions or the development of new lesions.

## Results

### Patients' characteristics

Five women and five men ranging in age from 47 to 72 years (median, 63 years) who had not previously been treated consented to enter the study. Their characteristics are given in Table 1. The primary localization site was the bladder in seven patients and the renal pelvis and the ureter in the three others. Six had a radical cystectomy and two received intravesical therapy with bacillus Calmette-Guérin (BCG).

### Pharmacokinetic dose adjustments

The two-compartment model described accurately the concentration versus time data. The linear regression between the 70 observed ultrafilterable carboplatin concentrations ($x$) and the concentrations obtained by pharmacokinetic analysis ($y$) was $y\ (\mu g/ml) = 0.99x + 0.11$ ($r = 0.997$). The pharmacokinetic results are summarized in Table 2. There was no significant difference (Students $t$-test for paired data) between the observed carboplatin clearance and the predicted clearance (GFR + 25), and the regression line was close to the identity line (Fig. 1). The delivered doses allowed us to obtain the target AUC ( ± 0.5 mg ml$^{-1}$ min) in 9/12 courses, and the difference between the expected AUC and the observed AUC never exceeded 1 mg ml$^{-1}$ min. The carboplatin clearance was compared with the creatinine clearance calculated according to the Cockroft-Gault equation [4]. Carboplatin clearance and (calculated creatinine clearance + 25) were poorly correlated ($r = 0.68$), and the regression line differed considerably from the identity line (Fig. 2).

**Table 1** Patients characteristics (*BSA* Body surface area)

| Patient | Age (years) | Sex | BSA ($m^2$) | Scr[a] | GFR[b] | Target AUC[c] |
|---|---|---|---|---|---|---|
| PAR | 67 | M | 1.87 | 122 | 93 | 3 |
| ZAN | 60 | M | 2.00 | 130 | 93 | 3 |
| VID | 52 | F | 2.00 | 128 | 95 | 3 |
| PIO | 72 | F | 1.43 | 75 | 49 | 4 |
| PUL | 68 | M | 1.64 | 158 | 54 | 4 |
| LAM | 47 | F | 1.54 | 64 | 91 | 4 |
| LAB | 55 | M | 1.98 | 216 | 48 | 5 |
| PRA | 68 | M | 1.72 | 182 | 31 | 5 |
| ZOR | 55 | F | 1.56 | 122 | 32 | 5 |
| LAY | 73 | F | 1.64 | 70 | 58 | 5 |

[a]serum creatinine level (μmol/l): normal range, 60–115 for men and 45–105 for women
[b]Glomerular filtration rate (ml/min) as measured by [$^{51}$Cr]-EDTA clearance [3]: normal range, 80–140 ml $min^{-1}$ 1.73 $m^{-2}$
[c]expressed in mg $ml^{-1}$ min

**Table 2** Pharmacokinetic parameters of ultrafilterable carboplatin (*Cmax* Maximal concentration, $t_{1/2}$ elimination half-life, *Vdβ* β-phase volume of distribution, *Cl* ultrafilterable carboplatin clearance)

| Patient, cycle | Dose (mg) | Cmax (mg/l) | $t_{1/2}$ (h) | Vdβ (l) | Cl (ml/min) |
|---|---|---|---|---|---|
| PAR, 1 | 355 | 15.1 | 3.0 | 25 | 111 |
| PAR, 2 | 385 | 15.0 | 3.2 | 29 | 99 |
| ZAN, 1 | 355 | 13.5 | 4.6 | 20 | 115 |
| VID, 1 | 360 | 15.0 | 6.9 | 50 | 95 |
| PIO, 1 | 292 | 13.8 | 2.3 | 17 | 97 |
| PUL, 1 | 316 | 16.1 | 2.9 | 17 | 77 |
| LAM,1 | 465 | 24.5 | 1.8 | 19 | 126 |
| LAM, 2 | 460 | 27.9 | 2.2 | 16 | 95 |
| LAB, 1 | 365 | 9.4 | 7.5 | 51 | 83 |
| PRA, 1 | 280 | 17.8 | 4.7 | 20 | 56 |
| ZOR, 1 | 285 | 16.1 | 4.6 | 20 | 56 |
| LAY, 1 | 415 | 27.6 | 3.5 | 17 | 75 |
| Mean ± SD | | | 4.2 ± 1.8 | 26 ± 13 | 89 ± 23 |

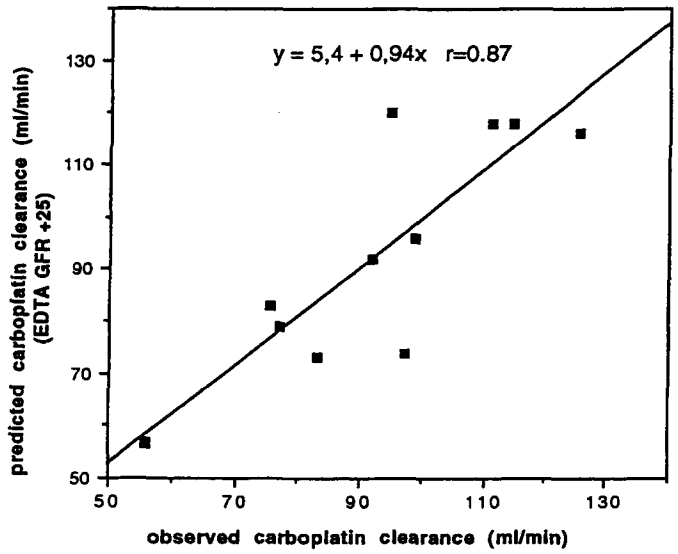


**Fig. 1** Relationship between the observed carboplatin clearance and the predicted value (GFR + 25), where GFR is the glomerular filtration rate determined by [$^{51}$Cr]-EDTA clearance measurement [3]. The line is given by linear regression analysis ($r = 0.87$, $P < 0.01$)

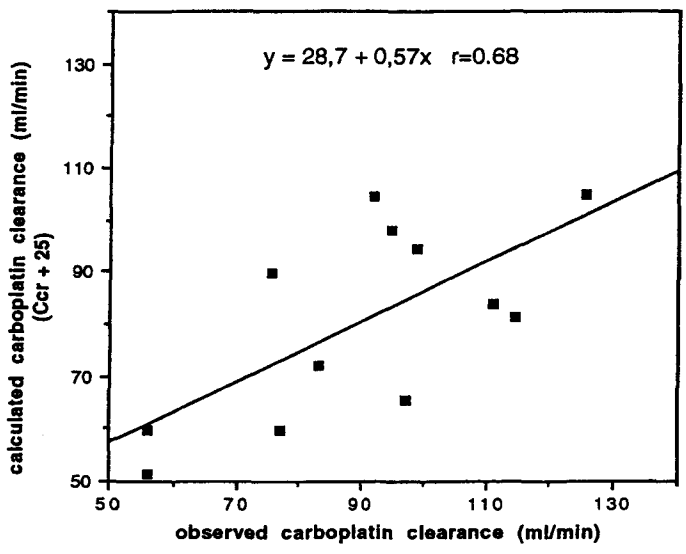


**Fig. 2.** Relationship between the observed carboplatin clearance and the value (Ccr + 25), where Ccr is the creatinine clearance calculated according to the Cockroft-Gault equation [4]: 1.23 × (140 − age) × weight/serum creatinine (μ*M*); for women, Ccr is 85% of the above value. The line is given by linear regression analysis ($r = 0.68$, $P < 0.05$)

Table 3 Neutropenia and thrombocytopenia versus the carboplatin AUC (*ANC* Absolute neutrophil count — neutropenia, *PT* platelet count — thrombocytopenia)

| Carboplatin AUC[a] | *n* | WHO grade 0 | | WHO grade 1 | | WHO grade 2 | | WHO grade 3 | | WHO grade 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ANC | PT | ANC | PT | ANC | PT | ANC | PT | ANC | PT |
| 2.5–3.5 | 3 | 1 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 |
| 3.5–4.5 | 5 | 0 | 4 | 1 | 0 | 1 | 1 | 3 | 0 | 0 | 0 |
| 4.5–5.5 | 4 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 2 | 0 |

[a]Observed AUC (mg ml$^{-1}$ min)

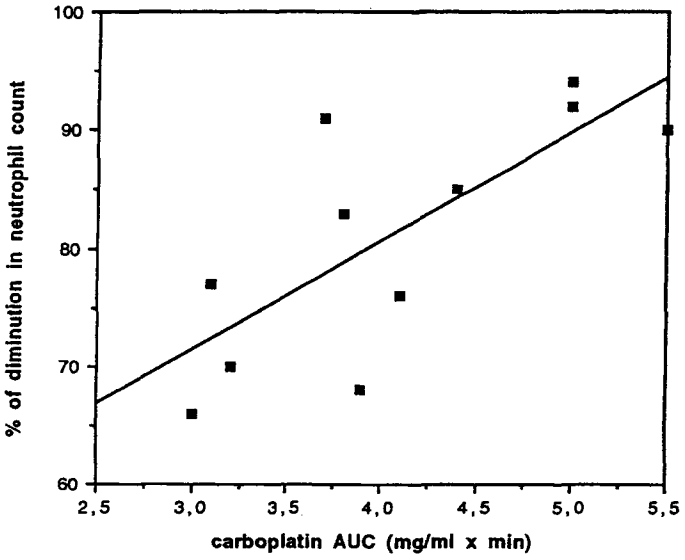


Fig. 3 Relationship between chemotherapy-induced neutropenia and the observed AUC for ultrafilterable carboplatin. The line is given by linear regression analysis ($r = 0.75$, $P < 0.01$)

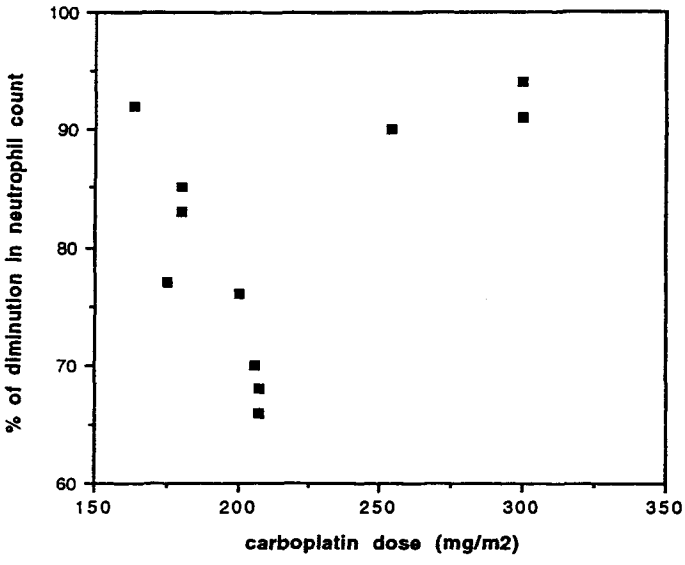


Fig. 4 Plot of chemotherapy-induced neutropenia versus the carboplatin dose (mg/m$^2$)

## Toxicity

Table 3 summarizes the hematological toxicity observed according to the carboplatin AUC. The dose-limiting toxicity was neutropenia. Episodes of thrombocytopenia were more moderate than those of neutropenia. The maximal tolerated AUC was 5 mg ml$^{-1}$ min, with 75% being of grade 3–4. Five of the eight second cycles given were delayed for 1 week due to neutropenia, including one of three, two of three, and two of two cycles given at AUC levels of 3, 4, and 5 mg ml$^{-1}$ min, respectively. Two cases of grade 3–4 mucositis were observed: one patient had a carboplatin AUC of 4 mg ml$^{-1}$ min and significant residual methotrexate levels at 24 and 48 h (0.60 and 0.16 $\mu M$, respectively); the other had a carboplatin AUC of 5 mg ml$^{-1}$ min, a methotrexate level of only 0.16 $\mu M$ at 24 h, and no residual methotrexate level at 48 h.

## Relationship between pharmacokinetics and toxicity

The percentage of reduction in the absolute neutrophil count was significantly correlated to the observed AUC: the best fit was obtained by linear regression analysis ($r = 0.75$, $P < 0.01$; Fig. 3). The use of sigmoidal models did not enhance the relationship. The percentage of reduction in the absolute neutrophil count was correlated neither to the carboplatin dose (in milligrams per square meter) [$r = 0.30$, not significant (NS); Fig. 4] nor to the residual methotrexate concentration ($r = 0.29$, NS). The percentage of reduction in the platelet count was correlated neither to the carboplatin AUC ($r = 0.30$, NS) nor to the residual methotrexate concentration ($r = 0.04$, NS).

## Response

Among seven assessable patients we observed one complete response (10 + months ; AUC level, 4 mg ml$^{-1}$ min),two cases of no change (7 + and 8 + months; AUC 3 and 4 mg ml$^{-1}$ min, respectively), and four cases of progressive disease (AUC levels, 3, 3, 4, and 5 mg ml$^{-1}$ min, respectively). The complete response was obtained in a patient who had a transitional-cell

carcinoma of the bladder; at diagnosis he had presented with prostatic invasion (pT4) and lomboaortic nodes, and he received five cycles of Carbo-MV. The evaluation by computerized tomography (CT) scan and the biopsy by cystoscopy done after the fourth cycle confirmed the complete response. The patient then received radiotherapy on the lomboaortic and pelvic area, and he currently remains in complete remission.

## Discussion

The GFR of the patients included in this trial confirm the great variability in the renal function of patients with urothelial cancer. Carboplatin is an alternative for patients with urothelial cancer who cannot tolerate cisplatin due to its nephrotoxicity. Adjusting the carboplatin dose according to the patient's renal function is now a well-established principle [8]. However, there are several limits to the use of pharmacologically based dosing of carboplatin.

First, carboplatin clearance needs to be predicted accurately. Our study confirms that the [$^{51}$Cr]-EDTA clearance method allows the investigator to obtain correctly the target AUC according to the Calvert formula [2]. The linear regression line between carboplatin clearance and (measured GFR + 25) had a slope of close to 1, and its intercept was only 5.4 ml/min. This isotopic method is resource-consuming, but it is the most reliable way to evaluate the GFR. Indeed, the use of creatinine clearance measurements can lead to an overestimation of GFR and, hence, to a risk of carboplatin overdosing [18]. Moreover, the creatinine clearance measurement is dependent on complete urinary recovery. An alternative to this measurement is calculation of the creatinine clearance (Ccr) using the Cockroft-Gault equation [4]. Our pharmacokinetic results show that this method is not accurate enough for use in adjusting the carboplatin dose. Indeed, Fig. 2 shows that the value (Ccr + 25) underestimated the carboplatin clearance in most (7/10) of the patients. The percentages of underestimation were as high as 29% and 33% for the patients ZAN and PIO, respectively, and there was no apparent explanation for this.

Second, the optimal carboplatin AUC needs to be determined for each combination chemotherapy used. The dose-limiting toxicity of carboplatin given in combination with other drugs is often leukopenia [7, 13, 15–17], and the target AUC for carboplatin must be decreased (e.g., 3–5 mg ml$^{-1}$ min for combinations with cyclophosphamide [15]). In our study, the dose-limiting toxicity was neutropenia (Table 3), as has also been described in other studies of the carboplatin, methotrexate, and vinblastine combination [1, 11]. The low degree of thrombocytopenia observed could explain the absence of correlation between the carboplatin AUC and the percentage of diminution in platelet count. Since the neutropenia was directly correlated with exposure to ultrafilterable carboplatin, we propose an AUC of 4 mg ml$^{-1}$ min as the target AUC for calculation of the carboplatin dose for the first cycle according to the formula *dose (mg)* $= 4 \times (GFR + 25)$. Indeed, at this AUC level, no grade 4 neutropenia was observed and the mean percentage of reduction in absolute neutrophil count was 80% ± 8%. At the AUC level of 5 mg ml$^{-1}$ min, two of four patients developed grade 4 neutropenia and the mean percentage of reduction in absolute neutrophil count was 92% ± 2%. It may be that in pretreated patients the target AUC for carboplatin dosing would be lower, in accordance with the results obtained in monochemotherapy [2, 5]. These results agreed with those obtained by Bellmunt et al. [1] in patients treated with Carbo-MV for bladder cancer; when these authors retrospectively estimated the carboplatin AUC from the creatinine clearance for their patients, a median value of 4.33 mg/ml was found.

This study confirms that the carboplatin dose should not be normalized using the body surface area, especially in patients suffering from impaired renal function. If a dose of 300 mg/m$^2$ had been given to the patients enrolled in our study, the AUC would have been 6.2 ± 1.6 mg ml$^{-1}$ min (range, 3.7–9.2 mg ml$^{-1}$ min) and probably would have been responsible for acute toxicity. A phase II trial of Carbo-MV, with the carboplatin dose being determined by the Calvert formula and using 4 mg ml$^{-1}$ min as the target AUC, is being planned to evaluate its dose intensity and its efficacy in patients with urothelial cancer.

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