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# A single-sample assay for the estimation of the area under the free carboplatin plasma concentration versus time curve

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**Abstract** The aim of this study was to develop and validate a simple and rapid method for the estimation of the area under the free carboplatin plasma concentration versus time curve (AUC). The relationship between the carboplatin AUC and the total plasma platinum (Pt) concentration 24 h after treatment was studied using data from 49 patients treated with 20–1600 mg/m$^2$ carboplatin as a 60–100 min infusion (median 60 min). The relationship was confirmed by the in vitro incubation of carboplatin in human plasma and prospectively validated in 13 ovarian cancer patients. Free carboplatin was separated by ultrafiltration (MW cut off 30,000), and free and total Pt measured by atomic absorption spectrophotometry. There was a linear relationship in vivo between the 24 h (median 24.4; range 16.3–27.3 h) total plasma Pt concentration ($\mu M$) and free carboplatin AUC (mg/ml.min): AUC =(24 h Pt +0.3)/0.82 ($r^2$ =0.93, AUC median 5.8 (0.13–28)mg/ml.min, 24 h Pt median 4.4 (0.1–23) $\mu M$). A similar relationship was observed in vitro [AUC =(24 h Pt + 0.1)/0.93 ($r^2$ =0.98, AUC median 7.9 (2.0–17) mg/ml.min, 24 h Pt median 7.1 (1.8–15) $\mu M$)]. The relationship derived from the in vivo data gave an unbiased and reasonably accurate estimate of the measured carboplatin AUC in 13 patients (AUC =5.1–8.7 mg/ml.min, GFR =59–129 ml/min, infusion time 30–45 min, 24 h sampling time 22.9–24.5 h), giving a percentage mean error of −4.2% and root mean squared percentage error of 11.5%. These results show that the analysis of a single blood sample taken 24 h after carboplatin administration can be used to produce an unbiased and reasonably accurate measure of the free carboplatin AUC. Unlike published limited sampling strategies, this method is not complicated by the need to accurately control the duration of the carboplatin infusion or the time at which the sample is taken.

**Key words** Carboplatin · Pharmacokinetics · Limited-sampling strategy · AUC estimation · Carboplatin AUC · Single-sample assay

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## Introduction

The pharmacokinetics of carboplatin, specifically the the area under the free carboplatin plasma concentration versus time curve (AUC), are major determinants of clinical toxicity and also, potentially, response [2, 5–8, 11]. Conventional pharmacokinetic studies to determine the carboplatin AUC require multiple blood sampling over the initial 6 h, at least, and ultrafiltration of plasma samples. This is an expensive and time-consuming practice, and in addition is inconvenient for the patient. The work described here was performed in order to develop a rapid and simple method for estimating carboplatin AUC values.

In a prospective study of 17 patients receiving cisplatin for cervical cancer, Fournier et al. [3] measured free and total Pt concentrations over the period 0–24 h after cisplatin administration. A linear correlation between the 0–24 h free Pt AUC and the total plasma Pt concentration 24 h after drug administration was demonstrated ($r$ =0.7, $P$ <0.01), which suggested that it might be possible to use the 24 h total plasma Pt concentration to estimate the 0–24 h free Pt AUC, although this possibility was not investigated prospectively by these authors.

Carboplatin is normally given as a 30–60 min infusion. Following the end of the infusion, plasma concentrations of total and free Pt decay biphasically with first order kinetics. Free carboplatin is cleared by renal elimination (predominant) and binding to macromolecules (minor) (reviewed by van der Vijgh [13]. By 24 h all of the free drug has been cleared and Pt in the

plasma is essentially totally and irreversibly protein bound. By analogy with the results of Fournier et al. [3], the binding of carboplatin to plasma proteins should be related to the concentration of the free drug available in the circulation. The aim of the work described in this study was to define the relationship between the free carboplatin AUC and the 24 h total plasma Pt concentration using both in vitro and in vivo data. Having defined the relationship, the bias and precision of the 24 h plasma Pt concentration in predicting the carboplatin AUC was assessed prospectively in 13 ovarian cancer patients.

## Materials and methods

### Study design

The study was performed in three stages. In the first part of the study, a retrospective analysis of the relationship between the in vivo free carboplatin AUC and the total plasma Pt concentration at 24 h was performed using data from 49 patients. From this retrospective analysis, an equation was developed to estimate carboplatin AUC (mg/ml.min) values from a single total plasma Pt ($\mu M$) determination performed on a sample taken 24 h after carboplatin administration. To confirm this relationship, in vitro experiments were performed using citrated human plasma and the relationship between the 0–24 h free Pt AUC and the bound 24 h Pt concentration was again investigated. Lastly, the equation developed from the in vivo data was validated prospectively in 13 patients with epithelial ovarian cancer who received carboplatin as a single agent.

### Measurement of Pt and carboplatin concentrations by atomic absorption spectrophotometry

Carboplatin concentrations in biological samples were determined using a Philips PU-9100X atomic absorption spectrophotometer (AAS; ATI Unicam, Cambridge, UK) adjusted to detect Pt (265.8–266 nm). The AAS was operated as a flameless furnace system. Table 1 details the temperature programme for the AAS used for plasma ultrafiltrate. The temperature and duration of the drying phase was varied according to the material tested; plasma needed a higher temperature (120°C) and longer drying time (60 s) than plasma ultrafiltrate. The expected sensitivity of the spectrophotometer was an absorbance of approximately 0.1A when a 20 µl aliquot of 58 ng Pt/ml 0.1 *M* HCl was analyzed. Blood samples (10 ml) were collected in heparinized tubes, and plasma was separated from cells by centrifugation (1200 g, 4°C, 10 min) within 15 min of blood collection. The protein-bound drug in the plasma was separated from the free drug by means of centrifugation (1200 g, 4°C, 15 min) through an ultrafiltration membrane with a molecular weight cut off of 30,000 by using Centrefree Micropartition units (Amicon, Beverly, Mass.). Plasma and plasma ultrafiltrate samples were either analyzed immediately or stored at −20°C for up to 2 weeks before analysis. Samples were diluted with 0.1 *M* HCl to bring their concentration to within the dynamic range of the spectrophotometer, 0.2–20 µg carboplatin/ml original fluid prior to dilution in 0.1 *M* HCl.

Prior to analysis by AAS, all samples and standards were diluted 1:20 (v/v) with 0.1 *M* HCl. Background absorption was determined with 0.1 *M* HCl and the AAS was calibrated using three standards. Carboplatin standard solutions were prepared prior to each run in 0.1 *M* HCl at 5, 10 and 20 µg carboplatin/ml, prior to dilution. The usual volume injected into the furnace was 10–20 µl and each sample was analyzed three times.

To determine interassay variation 10 µg carboplatin/ml in 0.1 *M* HCl, prepared and stored at −80°C, was measured once or twice depending on the assay size, during each run. All carboplatin concentrations refer to the intact parent drug (FW 371). Using the above quality assurance sample, intra-and interassay coefficients of variation were <10% and the measured carboplatin concentrations were within 15% of the nominal concentrations.

**Table 1** Temperature programme for the AAS analysis of Pt

| Phase | Temperature (°C) | Time (s) | Ramp (°C/s) |
|---|---|---|---|
| Drying | 100 | 40 | 5 |
| Ashing | 800 | 10 | 200 |
| Vaporization | 1100 | 10 | 200 |
| Atomization | 2700 | 3 | 0 |
| Cleaning | 2800 | 3 | 0 |

**Table 2** Characteristics of patients studied to define the relationship between carboplatin AUC and 24 h Pt concentrations in vivo

| | |
|---|---|
| Patients | |
| Total number | 49 |
| Female/male | 25/24 |
| Median age (range) (years) | 58 (17–78) |
| Carboplatin dose (mg/m$^2$) | 20–1600 |
| Tumur type | |
| Ovary | 19 |
| Lung | 11 |
| Connective tissue | 6 |
| Mesothelioma | 5 |
| Testicular | 3 |
| Hematological | 2 |
| Others | 3 |

### Retrospective analysis of the relationship between carboplatin AUC and 24 h total plasma Pt concentration

The relationship between the free carboplatin AUC and the total plasma Pt concentration approximately 24 h (median 24.4 (range 16.3–27.3) h, mean ± SD =24.0 ±1.9 h) after drug administration was established using data from 49 patients. Time points other than 24 h were not evaluated. These 49 patients had been treated as part of various studies which have been previously reported [1, 4, 8, 9] and represent all those patients for whom paired 0–24 h AUC and 24 h total plasma Pt data were available. All patients received single agent carboplatin as a 60 min infusion (median 60 (60–100) min) either on the basis of surface area ($n$ = 33; 20–1600 mg/m$^2$) or GFR ($n$ =16; target AUC 3–8 mg/ml.min) and the majority of the AUC values ($n$ =36) were within the range 3–10 mg/ml min. Renal function (GFR) in these patients was within the range 33–136 ml/min. Additional characteristics for these patients are summarized in Table 2.

### In vitro studies of the relationship between carboplatin AUC and protein binding

Carboplatin at six different concentrations (2, 4, 6, 8, 10 and 15 µg/ml) was incubated in duplicate in citrated plasma for up to

4 days at 37°C. Plasma was obtained from the National Blood Transfusion Centre, Newcastle Upon Tyne, UK. Five samples were taken at time points up to 90 h and in each sample the total and free Pt concentrations were measured by AAS. These data were used, as described below, to calculate the 0–24 h free carboplatin AUC and the bound plasma Pt concentration at 24 h for each incubation. In these in vitro studies free carboplatin was separated by ultrafiltration (as described above) or, for comparison, by trichloroacetic acid (TCA) precipitation involving the addition of an equal volume of 20% (w/v) TCA.

For each sample incubated, the total plasma Pt concentration was calculated as the mean of the five separate values obtained from the samples taken throughout the incubation. In every case the mean measured total plasma Pt concentration was within 10% of the nominal concentration. A monoexponential equation was fitted, using unweighted nonlinear least squares regression (GraphPAD Inplot version 3.04, San Diego, Calif.), to the free Pt concentration versus time data, namely:

$$C_t = C_0 e^{-kt} \quad \text{Eq.1}$$

where $C_t$ is the free Pt concentration ( μg/ml carboplatin equivalents) at time $t$ (h), $C_0$ is the extrapolated free Pt concentration at $t=0$ and $k$ is the first order rate constant for the binding of carboplatin to plasma macromolecules. The $C_0$ values so obtained were 2–15% lower than the mean total plasma Pt concentration, indicating that there was a low level of reversible binding of carboplatin to plasma macromolecules, as reported previously [4]. Using Eq.1 for each incubation, the free Pt plasma concentration at 24 h ($C_{24}$) was calculated and the bound Pt concentration at 24 h estimated as:

$$\text{Bound Pt (24 h)} = \text{Mean total Pt} - C_{24} \quad \text{Eq.2}$$

The 24 h bound Pt concentration was then corrected to account for reversible binding using the ratio of the extrapolated $C_0$ value to the measured mean total Pt concentration. The irreversibly bound 24 h Pt concentration so obtained was finally converted from micrograms per milliliter carboplatin equivalents to micromoles per liter Pt.

The 0–24 h free carboplatin AUC was estimated using the constants in Eq.1, and the calculated 24 h free Pt concentration, as follows:

$$\text{0–24 h free carboplatin AUC} = C_0/k - C_{24}/k \quad \text{Eq.3}$$

Thus, for each incubation, the irreversibly bound Pt concentration (μ*M*) at 24 h and the 0–24 h free carboplatin AUC (mg/ml.min) was estimated. The relationship between these two parameters was evaluated by linear regression analyses. One analysis was performed for data where free and bound Pt were separated by ultrafiltration and a second for samples where separation was by TCA precipitation. Comparisons were then made between these in vitro data and the retrospective in vivo data.

### Prospective evaluation of the performance of the 24 h plasma Pt concentration in predicting carboplatin AUC values

Patients with epithelial ovarian cancer who were receiving carboplatin as a single agent were eligible for entry into the study. The patients entered were those sequentially referred for treatment who consented to the pharmacokinetics studies. The clinical characteristics of these patients were median age 61 (range 44–75) years, surface area 1.7 (1.3–2) $m^2$ and WHO performance status 0–3. Serum total protein and albumin concentrations were within the normal range. None of the patients had a coexisting serious medical condition or abnormal renal function ($^{51}$Cr-EDTA clearance 59–129 ml/min). The dose of carboplatin was calculated either according to the pharmacokinetically guided formula described by Calvert et al. [1], target AUC 7 mg/ml.min ($n=8$), or according to the surface area, i.e. 400 mg/$m^2$ ($n=5$). Blood samples (10 ml) were collected in tubes containing heparin (10 IU/ml) prior to and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 12 and 24 h after a 30 min (median 35 (30–45) min) infusion of carboplatin. In these in vivo studies only ultrafiltration was used to separate free drug. The total plasma Pt and ultrafiltrable carboplatin concentrations were measured by AAS. The observed carboplatin AUC (mg/ml.min) was estimated using the trapezoidal rule and related to the AUC predicted from the single 24 h (median =24.0 (22.9–24.5) h) total plasma Pt sample assay developed above. The predictive performance (bias and precision) was assessed according to the method of Sheiner and Beal [10]. An information sheet was given to each patient and written consent was obtained prior to entry of any patient into the study. The approval of the Regional Ethics Committee was obtained prior to starting the study.

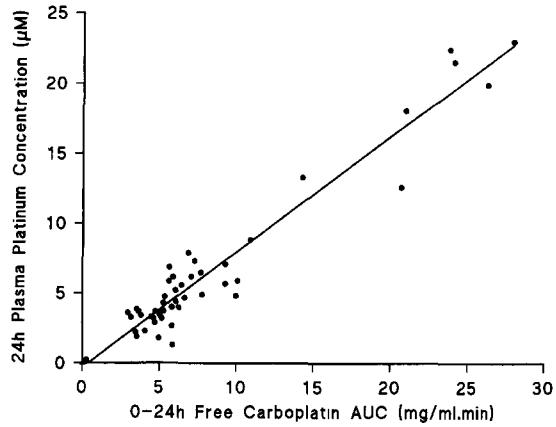


**Fig. 1** Relationship between 24 h total Pt concentration and carboplatin AUC in vivo. Each point represents an individual patient and the line is that given by linear regression analysis

## Results

### Retrospective analysis of the relationship between 0–24 h carboplatin AUC and 24 h total plasma Pt concentration in patients

The relationship between the 0–24 h free carboplatin AUC and the 24 h total plasma Pt concentration was studied retrospectively in 49 patients given carboplatin over a dose range of 20–1600 mg/$m^2$. These studies showed that by 24 h plasma Pt was present exclusively in the protein bound form and Fig. 1 demonstrates the result of the linear regression analysis of the relationship between 24 h total plasma Pt ( μ*M*) and 0–24 h free carboplatin AUC (mg/ml.min). There was a strong linear relationship between the 24 h total plasma Pt concentration and the 0–24 h carboplatin AUC ($r^2=0.93$) indicating that the amount of Pt bound to plasma protein at 24 h was primarily a linear function of the free drug exposure (0–24 h AUC). A residuals analysis was performed which showed that errors were not related to AUC and hence, by implication, dose (data not shown).

Reanalysis of the training data set, but with AUC values limited to the range 3–10 mg/ml.min ($n=36$)

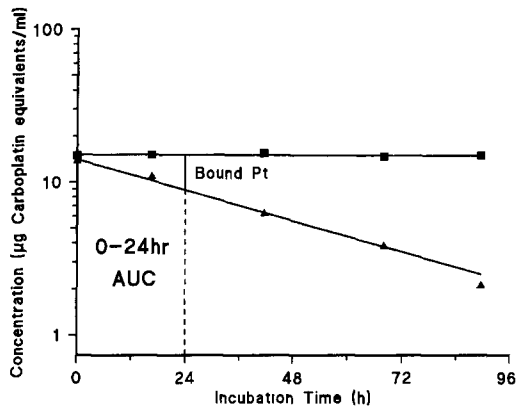


**Fig. 2** Total Pt (■) and free carboplatin (▲) concentrations following the incubation of 15 µg/ml carboplatin in human plasma at 37°C. Free and bound Pt were separated by ultrafiltration

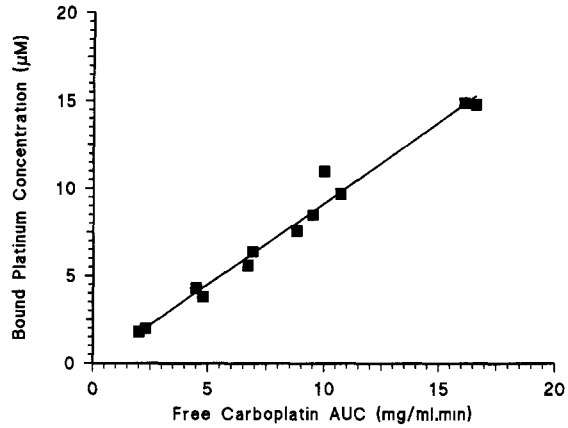


**Fig. 3** Relationship between 24 h total Pt concentration and carboplatin AUC in vitro with separation of bound and free Pt by ultrafiltration. Each point was derived from a separate incubation and the line is that given by linear regression

resulted in a regression analysis that was not significantly different from that given by the analysis of all 49 patients, i.e. 24 h Pt $=0.72(0.45-0.99)\times$ AUC $+0.31$ ($-1.2$–$1.87$) versus 24 Pt $=0.82$ (0.76–0.89) $\times$ AUC $-0.33$ ($-1.01$–$0.35$); (mean values and 95% confidence intervals). The coefficient of determination was considerably poorer for the limited range analysis ($r^2=0.46$ versus 0.93) and hence the regression equation derived from the analysis of all 49 patients was used to validate prospectively the use of the 24 h Pt concentration to estimate carboplatin AUC values.

**Table 3** Linear regression parameters for the relationship between 24 h bound Pt concentrations (µ*M*) and 0–24 h free carboplatin AUC (mg/ml min) following in vitro or in vivo exposure to carboplatin (*UF* ultrafiltration, *TCA* trichloroacetic and precipitation)

| Sample | Separation method | Slope ± SD (µ*M*/mg per ml.min) | Y intercept (µ*M*) | $r^2$ |
|---|---|---|---|---|
| In vitro | UF | 0.93 ± 0.15 | −0.1 | 0.98 |
| In vitro | TCA | 0.97 ± 0.14 | −0.3 | 0.98 |
| In vivo | UF | 0.82 ± 0.03 | −0.3 | 0.93 |

## Relationship between carboplatin exposure and the in vitro plasma protein binding of carboplatin

Carboplatin was incubated at 37°C with human plasma at a range of concentrations (2–15 µg/ml) so as to achieve 0–24 h free carboplatin AUC values within the range seen in patients. In addition to using ultrafiltration to separate free from bound carboplatin, precipitation with 20% (w/v) TCA 1:1 (v/v) was evaluated. In these in vitro experiments, as the main factor affecting free carboplatin loss was expected to be binding to plasma proteins, the total plasma protein and albumin concentrations were measured in all samples and found to be 69–85 and 41–46 g/l, respectively, i.e. within normal ranges.

Figure 2 shows an example of total Pt and free carboplatin concentrations with time when 15 µg/ml carboplatin was incubated in human plasma for 90 h and ultrafiltration was used to separate free from bound Pt. The correlation between the 24 h bound Pt concentration and 0–24 h free carboplatin AUC is shown in Fig. 3. The results obtained using TCA to separate free carboplatin and bound Pt were comparable to those obtained using ultrafiltration. Thus for both ultrafiltration and TCA precipitation there was a strong and highly significant relationship between the bound plasma Pt concentration at 24 h and the 0–24 h free carboplatin AUC. The regression equations for these in vitro relationships are given in Table 3 and compared with the result from the retrospective analysis of clinical data.

## Prospective evaluation of the use of 24 h plasma Pt concentrations to predict 0–24 h carboplatin AUC values

Model independent analysis of carboplatin pharmacokinetics in the 13 patients treated with either an AUC of 7 mg/ml.min or 400 mg/m$^2$ carboplatin revealed an AUC of 7.2 ± 1.3 mg/ml.min (mean ± SD), median 7.5 (5.1–8.7). The AUC was also estimated for each patient from the 24 h total plasma Pt concentration using the relationship:

$$\text{AUC (mg/ml.min)} = \frac{\text{24 h Pt}(\mu M) + 0.3}{0.82}$$

Figure 4 demonstrates the relationship between the observed and predicted AUC values. Using the 24 h Pt concentration, the bias (mean prediction error ±SEM, MPE) was $-0.36 \pm 0.23$ mg/ml.min, and the precision (mean squared prediction error, MSE, and root mean

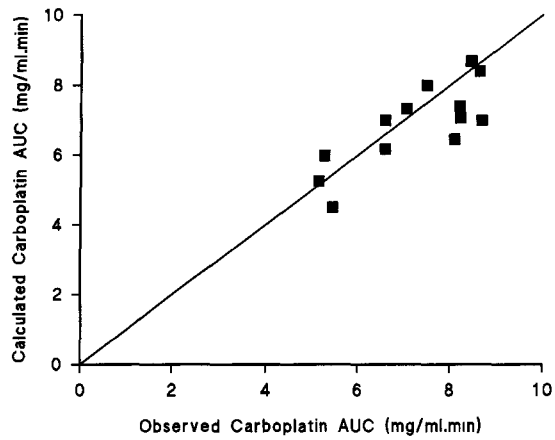


**Fig. 4** Relation between observed carboplatin AUC values and those estimated from the 24 h total plasma Pt concentration according to the equation AUC (mg/ml.min) = [24 h Pt ($\mu M$) + 0.3]/0.82. The line is the line of identity

squared prediction error, RMSE) were 0.75 (mg/ml.min)$^2$ and 0.87 mg/ml.min, respectively. Thus the percentage mean prediction error (MPE% ±SEM) and the root mean squared percentage error (RMSE%) were −4.2 ±3.1% and 11.5%, respectively.

## Discussion

This study demonstrates that a single blood sample taken 24 h after carboplatin administration can be used to produce an unbiased and reasonably accurate estimate of the 0–24 h carboplatin AUC (MPE% −4.2% and RMSE% 11.5%). In comparison, interpatient variation in carboplatin clearance, and hence AUC when dosing is performed on the basis of surface area alone, is in the region of 300% (reviewed by van der Vijgh [13]). In vitro studies were performed to confirm the relationship initially observed in vivo and these again demonstrated an excellent correlation between the 0–24 h AUC and the 24 h Pt concentration.

Sørensen et al. [12] have also developed a limited sampling method for the estimation of carboplatin AUC based on stepwise regression analysis in which the free carboplatin AUC is related to the free carboplatin concentration at a single time-point is from within the range 0.25–10 h after the end of the drug infusion. The optimal single sampling time-point is 2.75 h and the free carboplatin AUC is given by the formula:

$$\text{AUC (mg/ml.min)} = 0.52 \times C_{2.75h} + 0.92$$

where $C_{2.75h}$ is the free carboplatin concentration (μg/ml) 2.75 h after the administration of carboplatin as a 60 min infusion. The above single sample formula was validated prospectively in nine patients; the MPE% was 4.4% and the RMSE% was 13.9%. To improve the accuracy of the formula, stepwise multiple linear regression analysis was performed which resulted in a second formula requiring two blood samples, one at 0.25 h and one at 2.75 h:

$$\text{AUC} = 0.053 \times C_{0.25h} + 0.401 \times C_{2.75h} + 0.628$$

This two-sample formula more accurately predicted the observed AUC; MPE% and RMSE% were 2.2% and 9.4%, respectively. More recently, the single-sample method proposed by Sørensen et al. [12] has been independently validated by van Warmerdam et al. [14] who found that the single sample taken at 2.75 h did give an unbiased and precise estimate of the carboplatin AUC in nine patients (MPE% −3.4%, RMSE% 5.2%). Although the latter results may compare favourably with those described here, in the approach developed by Sørensen et al. the duration of carboplatin infusion must be fixed at 60 min and the timing of the collection of the one or two samples must be accurate. In contrast, in the formula developed here, the duration of carboplatin infusion or the exact time of the 24 h blood sample should not markedly alter the accuracy of the AUC estimation. In the present study, the median and ranges of the infusion and sampling times were 60 (60–100) min and 24.4 (16.3–27.3) h, respectively, for the training data set and 35 (30–45) min and 24.0 (22.9–24.5) h, respectively, for the validation data set. By 24 h, Pt in the plasma is completely protein bound and the protein-bound Pt has a half-life of more than 5 days (reviewed by van der Vijgh [13]). Deviation from the 24 h time point by 1 h or 2 h therefore has little effect on the observed Pt concentration. Similarly, as the 24 h plasma Pt concentration is dependant upon the carboplatin AUC and not the peak concentration, the duration of the infusion will not markedly affect the result, providing the duration of infusion does not approach 24 h.

Carboplatin pharmacokinetics are markedly dependent on renal function and, in the present study, 59 ml/min was the lowest GFR in the group of patients in whom the single-sample method was evaluated prospectively. Theoretically, in patients with very poor renal function, free carboplatin could still be present in the plasma at 24 h. In such cases ultrafiltration would be required to correct the total plasma Pt concentration for residual free drug prior to the estimation of the 0–24 h free carboplatin AUC from the 24 h plasma Pt concentration.

In addition to the prospective clinical evaluation of the single-sample 24 h bound Pt assay, in vitro studies were performed to confirm the relationship between carboplatin exposure and the binding of Pt to plasma proteins. These in vitro studies confirmed that there was a very strong linear relationship, but the slopes of the in vitro relationships were slightly greater than those derived from in vivo data. In the in vitro experiments parent carboplatin concentrations declined solely by binding to plasma proteins which are not removed from the system. The limited clearance of

platinated proteins in vivo during the 24 h period of the study may explain the slightly lower slope value for the relationship between 24 h bound plasma Pt concentration and the 0–24 h free carboplatin AUC in vivo compared to that found in vitro. Alternatively the use of citrate, as opposed to heparin, as the anticoagulant in the in vitro experiments may have led to increased carboplatin binding by enhancing either the reactivity of the carboplatin or the plasma proteins.

In the in vitro studies both ultrafiltration and TCA precipitation were used to separate bound from free carboplatin. There was no difference between the methods and either could be used to calculate the carboplatin AUC. The TCA precipitation method would be particularly useful in preclinical or pediatric studies where only small volumes of blood are available.

In summary, the method described in this paper provides an unbiased and reasonably accurate estimate of the free carboplatin AUC achieved in patients. It has advantages over either full pharmacokinetic monitoring and published limited ultrafiltrable-Pt sampling strategies in that it is simple, flexible and less time consuming, although the collection of a blood sample at 24 h is required. As demonstrated here, the method can be used to measure carboplatin AUC values after conventional doses and it has also been used in patients receiving high AUC intensity therapy.

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