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Dose-toxicity relationship of carboplatin in combination with cyclophosphamide in ovarian cancer patients*

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Summary. A study was undertaken to examine the relationships between carboplatin's pharmacokinetic parameters and the myelotoxicity associated with its administration in combination with cyclophosphamide. An additional aim of the study was to test the applicability of the method proposed by Calvert et al. for calculation of the carboplatin dose to be used in the combination regimen. A total of 24 previously untreated ovarian cancer patients were given a combination of 250–500 mg/m^2 carboplatin and 500 mg/m^2 cyclophosphamide every 4 weeks for 4 months. The pharmacokinetics of carboplatin and the associated myelotoxicity were investigated in 64 courses. The results showed a significant correlation ($r = 0.89$) between the AUC calculated for carboplatin and that predicted according to Calvert's formula [carboplatin dose in milligrams = AUC (glomerular filtration rate +25)]. We conclude that the model is a useful guide in the calculation of the carboplatin dose to be given in combination with cyclophosphamide, and it enables a more precise prediction of the carboplatin exposure than does the conventional calculation, which is based on milligrams of drug per square meter of body surface. The AUC for carboplatin was a reliable predictor of the myelotoxicity as measured by the relative decrease in thrombocyte count. However, the relationship between AUC and myelotoxicity changed during the treatment because of increasing bone marrow toxicity. Despite this finding, dose calculation based on carboplatin's AUC appears to provide an improvement in the clinical use of the drug, and the method also seems to be fully applicable in combination chemotherapy with cyclophosphamide.

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Introduction

Carboplatin probably equals cisplatin in terms of spectrum and tumouricidal effect, but the former causes many fewer subjective and chronic side effects such as nephrotoxicity, neurotoxicity, and ototoxicity [5, 18]. Consequently, carboplatin is a candidate for the replacement of cisplatin in chemotherapy. The dose-limiting side effect of carboplatin is myelotoxicity, with thrombocytopenia dominating leukopenia. Impairment of renal function is reported to be associated with carboplatin retention and, thus, with an increased risk of myelotoxicity. Measurement of the patient's renal function is therefore important in determination of the carboplatin dose [10, 16, 17].

Several studies have been carried out to develop methods for individual carboplatin-dose calculation [6, 9–11, 14, 21]. Assuming that the AUC is an important predictor of the myelotoxic effect, Calvert et al. [6] have suggested a formula by which the carboplatin dose is calculated on the basis of a target AUC and the glomerular filtration rate (GFR) measured by [^{51}Cr]-ethylenediaminetetraacetic acid (EDTA) clearance. Egorin et al. [10, 11] developed another formula for calculation of the carboplatin dose per square meter of body surface that is based on an acceptable relative decrease in the thrombocyte count and the pretreatment creatinine clearance. These studies concentrated on the administration of carboplatin as a single agent. In combination treatment, the myelotoxicity may be expected to increase due to the additive effect produced by the administration of two or more drugs. Thus, the next logical step is to investigate whether the individual dose-calculation method can be applied when carboplatin is given in combination with other cytostatic agents. The paper by Belani et al. [2, 3, 20] on the combination of carboplatin and etoposide is the only report thus far published on this topic.

The clinical observation that bone-marrow sensitivity can be increased in patients who have received prior chemotherapy has been confirmed by Egorin et al. [10], who reported that the reduction in the platelet count in pretreated patients was 17% higher than that in non-pretrated

Table 1. Patients' characteristics

Number of patients	24
Median age (range)	54.5 (39–67) years
Mean WHO performance status (range)	1.3 (0–3)
Residual disease:	
≤1 cm	6
>1 cm, ≤5 cm	5
>5 cm	13
Mean [^{51}Cr]-EDTA clearance (range)	86.6 (49.3–120.9) ml/min

patients at a given AUC level. However, the extent to which this finding should be taken into consideration in calculations of the carboplatin dose for combination chemotherapy is not clear.

Recent clinical trials have suggested that carboplatin may replace cisplatin in ovarian cancer therapy, and there has thus far been no report of any significant difference in antitumour activity between the cisplatin/cyclophosphamide and the carboplatin/cyclophosphamide regimens [1, 9, 22]. As the latter regimen causes fewer chronic side effects, it will probably be preferred by many centres in the future.

Our aims were to characterize the relationship between the pharmacokinetic characteristics of carboplatin given in combination with cyclophosphamide and the associated myelotoxicity (changes in the thrombocyte count), to evaluate the applicability of Calvert's formula in this combination regimen, and to analyse the effect of the number of courses given on the sensitivity of the bone marrow.

Patients and methods

A total of 24 patients were allocated to the pharmacokinetic study; their characteristics are summarized in Table 1. The inclusion criteria were as follows: histologically verified epithelial ovarian carcinoma of FIGO (International Federation of Gynecology and Obstetrics) stages Ic-IV, no prior treatment, an age of ≤70 years, and normal bone-marrow function (leucocyte count, $>3\times10^9$/l; thrombocyte count, $>150\times10^9$/l). Informed consent was obtained from all subjects.

In all, 23 of the patients exhibited a pretreatment [^{51}Cr]-EDTA clearance of >60 ml/min and were randomized to receive 250, 375, or 500 mg/m^2 carboplatin. One patient showing a [^{51}Cr]-EDTA clearance of 49.3 ml/min was given 250 mg/m^2 carboplatin. All patients received 500 mg/m^2 cyclophosphamide.

Cyclophosphamide was diluted into 75 ml isotonic saline and given as a 15-min i.v. infusion. Carboplatin was diluted into 300 ml isotonic saline and infused over 1 h immediately after the cyclophosphamide infusion. The infusion rate was held constant by an IVAC infusion pump (model 281; IVAC Corporation, San Diego, Calif.) The treatment was repeated every 4 weeks for 4 months. Haemoglobin, the leucocyte count, and the thrombocyte count were measured weekly between treatments. [^{51}Cr]-EDTA clearance [4] was determined before each chemotherapy course.

In patients exhibiting a leucocyte nadir of $<1\times10^9$/l or a thrombocyte nadir of $<50\times10^9$/l, the subsequent doses of carboplatin were reduced by one step, i.e. from 500 to 375 mg/m^2, from 375 to 250 mg/m^2, or from 250 to 200 mg/m^2. Treatment was postponed for 1 week if the leucocyte count was $<3\times10^9$/l or the thrombocyte count was $<150\times10^9$/l on the day of treatment.

Pharmacokinetic studies were planned at the first, second, and fourth chemotherapy courses. Blood samples for carboplatin analysis were collected in tubes containing sodium heparin both prior to and at the following times after carboplatin infusion: 0.05, 0.25, 0.75, 2.75, 3.75, 4.75, 6.75, 8.75, and 22 h. The blood samples were immediately cooled, centrifuged, and stored at –70° C until analysis.

Determination of the carboplatin concentration in the plasma ultrafiltrate and the urine was carried out using the high-performance liquid chromatography (HPLC) method previously described by Duncan et al. [8]. Intra-day and inter-day coefficients of variation were 5.3% and 7.7%, respectively, at 10 μg/ml; the detection limit was 0.2 μg/ml and the coefficient of variation was <25%.

Using the computer program GraphPad Inplot (GraphPAD Software, San Diego, Calif.), post-infusion carboplatin plasma concentration versus time curves were fitted to a biexponential equation, assuming a two-compartment model for the distribution and elimination of carboplatin [13]:

$c(t) = A' \times e^{-\alpha t} + B' \times e^{-\beta t}$,

where c is the concentration at time t, A′ and B′ are concentration constants, and α and β are rate constants. The AUC from the beginning of the carboplatin infusion to infinity was calculated using the following equation [15]:

$$AUC_{0-\infty} = \frac{A'T}{1-\exp(-\alpha T)} + \frac{B'T}{1-\exp(-\beta T)},$$

where T represents the infusion time.

Using linear regression analysis, the observed AUC was compared to the predicted AUC calculated using the formula of Calvert et al. [6]:

Dose (mg) = AUC (GFR +25).

The calculation of the predicted AUC was based on the delivered dose and on the pretreatment [^{51}Cr]-EDTA clearance actually measured.

We chose the relative decrease in the thrombocyte count (expressed in percentage) as a measure of bone-marrow toxicity, because thrombocytopenia is the major and dose-limiting side effect of carboplatin. Sensitivity to carboplatin treatment was defined as the relative decrease in the thrombocyte count divided by the carboplatin AUC, assuming that the cyclophosphamide treatment at a given dose level plays a negligible role in the development of thrombocytopenia [7]:

$$\text{Sensitivity} = \frac{\text{Relative decrease in thrombocyte count (\%)}}{\text{Carboplatin AUC (mg ml}^{-1}\text{ min)}},$$

The sensitivity was individually calculated for each patient and for each of the courses studied. Individual values for bone-marrow sensitivity calculated from the first, second, and fourth courses were compared using Student's paired t-test. A significance level of 5% was applied in all cases.

Results

The combination of carboplatin and cyclophosphamide was well tolerated by the patients. Thrombocytopenia was the main dose-limiting factor. The thrombocyte nadir ranged from 9 to 280×10^9/l (mean, 106×10^9/l), and the leucocyte nadir ranged from 0.7 to 4.2×10^9/l (mean, 2.3×10^9/l). Two courses had to be delayed because of leucopenia. In five cases, the carboplatin dose was reduced according to the protocol; in four, due to low thrombocyte nadirs; and in one, because of a low leucocyte nadir. Two patients who developed thrombocytopenia exhibited petechiae but no mucosal bleeding. None of the patients developed treatment-related infections, and there were no treatment-related deaths. Emesis was mild to moderate and was easily treated with antiemetics.

The relationship between the carboplatin dose per square meter of body surface and the measured AUC is shown in Fig. 1. It is obvious that an increase in the dose tended to cause a higher AUC, but the latter varied up to 2-fold at any given dose level. Figure 2 compares the AUC values determined in the present study with those calcu-

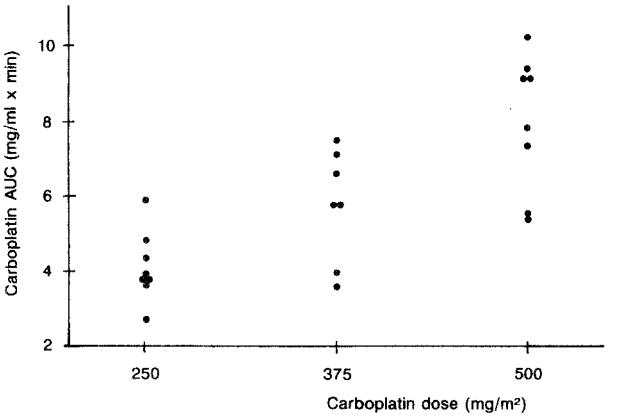

Fig. 1. Relationship between the carboplatin AUC and the dose as determined at the 3 different dose levels used in the first course ($n = 23$)

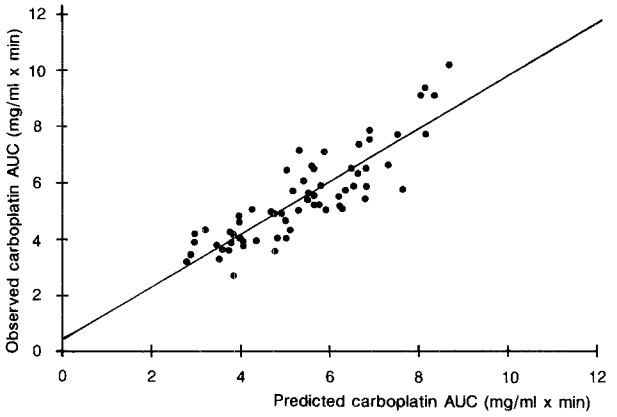

Fig. 2. Correlation of the observed and the expected carboplatin AUC as calculated according to the formula Dose (mg) = AUC $\times$(GFR +25). The linear regression line is shown. The mean value $\pm$ SE for the slope is 0.93 ± 0.07 and that for the intercept is 0.43 ± 0.37 ($r = 0.87$)

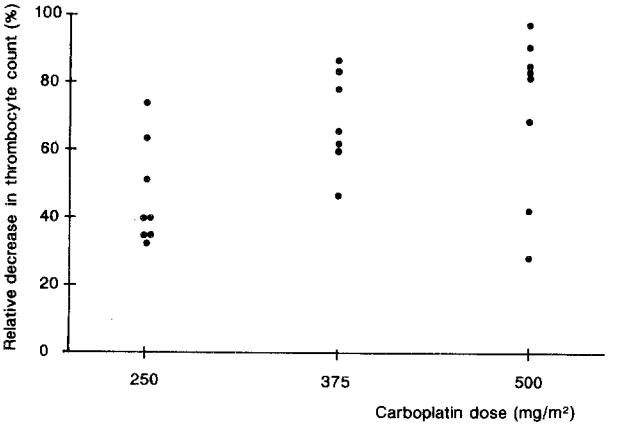

Fig. 3. Plot of the carboplatin dose given in the first course vs the relative decrease in thrombocyte count

lated using Calvert's formula; it includes the results obtained for all courses and demonstrates a significant relationship ($r = 0.89$, $P < 0.00001$). The slope of the regression line was not significantly different from unity.

The relationship between the carboplatin dose calculated in milligrams per square meter of body surface and

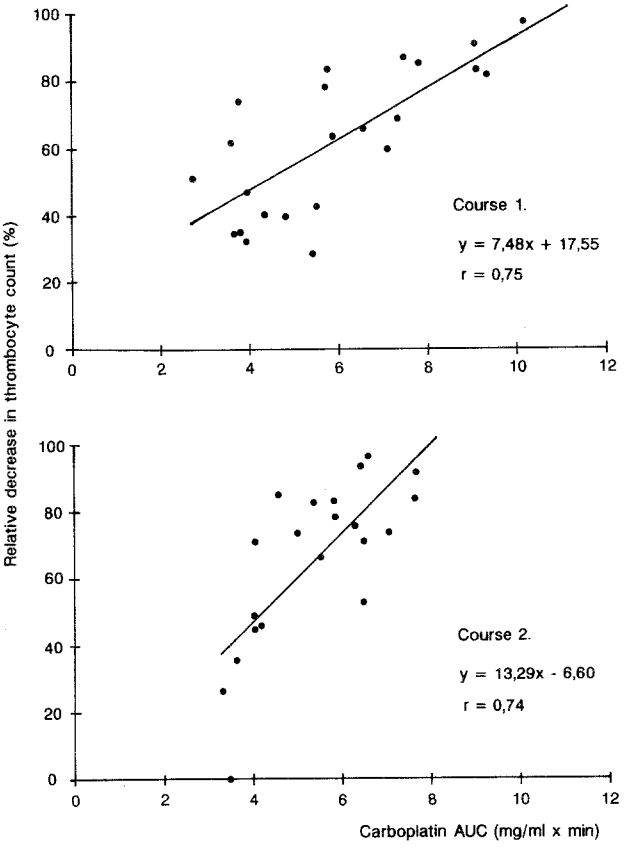

Fig. 4. Carboplatin AUC vs the relative change in thrombocyte count for the first (*upper panel*) and second courses (*lower panel*) Corse 1: $y = 7.48x + 17.55$, $r = 0.75$; course 2: $y = 13.29x - 6.6$, $r = 0.74$

the relative decrease in the thrombocyte count is shown in Fig. 3. The inadequacy of the carboplatin dose calculated thusly as a measure of the treatment exposure is obvious from the results, because the decrease in the thrombocyte count varied by a factor of >2 at any dose level.

Figure 4 compares the relative decrease in the thrombocyte count with the carboplatin AUC at the first and second courses; linear regression analyses indicated correlation ($r_1 = 0.75$, $r_2 = 0.74$). It is clear that the bone-marrow sensitivity appeared to increase (slope 1, 7.48; slope 2, 13.29). The same tendency was observed at the fourth course, for which the slope was 18.01 (data not shown). Further statistical analysis of the bone-marrow sensitivity data using Student's paired *t*-test revealed no significant difference in sensitivity between the courses when all patients were included, but subjects ($n = 14$) achieving an AUC value of ≥ 4 exhibited a highly significant increase in sensitivity from the first course to the second ($P = 0.0001$). No significant difference in sensitivity was found between the second and the fourth courses.

Discussion

Cytostatic dose calculations are often based on patients' body surface area, which is considered to correlate well with the metabolic rate and the ability to eliminate cytostatic agents. Experience has shown that this method of

dose calculation is feasible for many drugs. On the other hand, severe toxic effects are often seen during chemotherapy, and it is quite clear that dose calculation based on the surface area of the patients alone is by no means ideal for all cytostatics. This problem attracts considerable interest because the toxic side effects may be severe or even fatal. As a relatively new drug, carboplatin has a wide range of potential uses; however, these need to be further elucidated.

Renal filtration is the major route of elimination and should be accounted for in dose calculations [6, 9–11, 14, 21]. Previous studies by Calvert et al. [6] have clearly demonstrated a simple relationship between the total dose of carboplatin and both the AUC and GFR, and these authors purport that their formula should replace that used in conventional dose calculation, which is based on the patient's body surface area.

The results of the present study indicate that Calvert's formula can also be applied when carboplatin is given in combination with cyclophosphamide; furthermore, a simple relationship seems to apply over a wide range of doses, which is mandatory for the practical use of the formula. The absence of systematic differences in carboplatin clearance values among the first, second, and fourth courses suggests that the pharmacokinetic characteristics of carboplatin are independent of previous dosing.

The crucial question is whether the carboplatin AUC is an adequate predictor of the pharmacodynamics of the drug. The present results indicate that there is a simple linear relationship between the AUC and the relative percentage of decrease in the thrombocyte count over a relatively wide and clinically relevant range of AUC values (4–10 mg ml^{-1} min). One should expect a deflection of the line at higher AUC values because the relative percentage of decrease in the thrombocyte count cannot by definition be >100%. For other cytostatics, the relationship between the AUC and the relative percentage of decrease in the platelet count has been described by the following equation [12]: % of decrease in thrombocyte count = $100 (1 - e^{-kxAUC})$. It seems possible that such a model would also apply to the relationship between the carboplatin AUC and the relative thrombocyte count. However, a wider range of AUC values must be investigated to test this hypothesis.

The sensitivity of the bone marrow must be taken into account when the AUC is used for dose calculation. In the present study, an increase in sensitivity occurred as early as at the second course for carboplatin AUC values of ≥ 4 mg ml^{-1} min. Thus, an AUC value tolerated during the first course without serious complications may result in considerable thrombocytopenia in the second or subsequent courses.

The present study was carried out in patients treated with both cyclophosphamide and carboplatin. The results indicate that in this combination regimen as well, the carboplatin AUC is a better tool for dose calculations than is the body surface area. A simple formula that would be applicable in all situations is not available, as the linear relationship between the AUC and the relative decrease in the thrombocyte count was demonstrated within only a limited range of AUC values in the present investigation. Furthermore, the bone-marrow sensitivity increased significantly with increasing numbers of chemotherapy courses. Nevertheless, dose calculations based on the AUC value seems to provide a major improvement in the clinical use of carboplatin because they render the treatment more safe and reduce the risk of underdosing while simultaneously improving the prospect for a more detailed study of the exposure-response relationships in these regimens.

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