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ORIGINAL ARTICLE

Pharmacokinetics of carboplatin and etoposide in infant neuroblastoma patients

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Abstract
Purpose Carboplatin and etoposide are commonly used chemotherapeutics for the treatment of many paediatric cancers. However, there are very limited published data concerning the pharmacokinetics of these agents in infants and very young children, for whom dose reductions are frequently implemented.
Methods Etoposide (5 mg/kg; 2 h i.v. infusion) was co-administered with carboplatin (6.6 mg/kg; 1 h i.v. infusion) on each of 3 days of treatment and samples were taken between 0.5 and 4 h after the start of administration, from a total of 19 neuroblastoma patients aged <1 year at diagnosis and weighing <12 kg at treatment. Pharmacokinetic analysis was carried out using a non-linear mixed effects modelling approach.
Results Two compartment structural models were selected for both carboplatin and etoposide analysis. Body weight (BW) was strongly associated with carboplatin clearance (Cl), with a slightly weaker relationship observed with etoposide Cl. Carboplatin Cl values ranged from 12.8 to 33.6 ml/min, with total AUC values of 4.2–9.3 mg/ml.min achieved over the 3 days of treatment. Cl values normalized to BW were significantly higher in patients <12 kg than in children >12 kg, with mean ± SD values of 2.9 ± 0.4 and 2.5 ± 0.4 ml/min/kg, respectively ($P < 0.05$). Etoposide exhibited a median half-life of 4.6 h (range 4.1–6.6), a median AUC of 7.1 mg/ml.min (range 3.4–11.0) and a median Cl of 6.6 ml/min (range 3.2–13.0).
Conclusion Results suggest that prediction of absolute carboplatin Cl values may be difficult in infant patients <12 kg, with a small but significant difference in Cl values normalized to BW observed in this patient group. Etoposide pharmacokinetic data support previous findings that question the utility of modified dosing in infants. The current study demonstrates the feasibility of generating informative pharmacokinetic data in infants and young children.

Keywords Carboplatin · Etoposide · Pharmacokinetics · Paediatrics · Infants · Neuroblastoma

On behalf of the CCLG Pharmacology Working Group.

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Introduction

The cytotoxic drugs carboplatin and etoposide are commonly used components of multi-drug chemotherapy for the treatment of a large number of paediatric cancers including neuroblastoma, soft tissue sarcomas, brain tumours and leukaemia [1–3]. Despite having been developed several decades ago, and although their utility is associated with numerous side effects in many patients [4], they are likely to maintain a key role in the treatment of childhood cancer for the foreseeable future.

Carboplatin and etoposide are administered concomitantly by intravenous infusion over 1–4 h in the majority of patients. However, a comparison of the dosing regimens for these drugs demonstrates that instructions for dosing in infants and very young children frequently differ between tumour types and protocols without scientific rationale. Indeed, this is common with many other standard cytotoxic chemotherapeutic drugs used in paediatric oncology [5]. Dose may be based on body surface area (SA) in one disease and on weight in another for the same age infant or child or, alternatively, the dosage reduction for a younger child may be modified based on body size in one protocol and age in a similar study for a different tumour type. In addition, the use of cut-off points to define dosing in 'infants' results in significant variations in the dose of drug administered to patients on either side of the defined cut-off point, despite similarities in physical characteristics.

Although the most common approach is to shift from standard SA-based dosing to dosing based on body weight (BW) in infants, further empirical dose reductions are often implemented, such as a 33 or even 50% decrease. The effect of these dosing approaches is to significantly alter the dose of chemotherapy given to infants and young children treated on the same protocol, with the potential for the administered dose to change dramatically as a child crosses an age or weight boundary during a multiple course chemotherapy regimen.

As there are currently very limited published data concerning the pharmacokinetics of the vast majority of anticancer drugs in infants, there is minimal scientific input into how these agents are dosed in the clinic. Given the toxicity observed with many of the cytotoxic drugs commonly used in infants and young children, it would seem appropriate to investigate how these drugs are dosed, why these dosing parameters have been chosen, and whether a more rational, evidence-based approach to dosing chemotherapy drugs in infants and young children can be established. This type of approach, based on an improved understanding of pharmacokinetics, may lead to clinical benefits for many of the drugs used in paediatric oncology for the treatment of young children [6]. The need for an increased availability of information on the use of medicines in children led to the introduction of the FDA Pediatric Final rule in the US and has recently been highlighted by the European Paediatric Regulation which came into force in January, 2007. It is anticipated that such regulations will lead to an increased knowledge of pharmacokinetics, efficacy and safety both in newly developed drugs as well as medicinal products currently used in a paediatric patient population [7]. In order to facilitate research in this area, the European Medicines Agency (EMEA) produced a list of off-patent drugs deemed as representing those products where there is currently the greatest need for additional paediatric information. This list, produced following consultation with experts in paediatric medicine, includes specific requests for data on pharmacokinetics of carboplatin and etoposide in children <2 and 3 years of age, respectively.

The current study was designed to obtain pharmacokinetic data for the cytotoxic drugs carboplatin and etoposide in neuroblastoma patients <1 year of age at diagnosis, a patient subgroup in which there is very limited prior knowledge of drug pharmacokinetics. Patients were treated on the European Infant Neuroblastoma Study (INES) protocol, receiving standard doses of carboplatin and etoposide as determined by BW. The main aim of the study was to determine plasma drug concentrations and the extent of inter-patient variation in pharmacokinetics in this patient group. These data could then be compared to those previously obtained in older children, in order to give an insight into the relative exposure of infant patients to these drugs at the doses currently indicated. Correlating drug exposure with toxicity and clinical response may allow a more rational approach to the treatment of infant patients with carboplatin and etoposide in future studies.

Materials and methods

Patient eligibility and treatment

The study protocol was approved by the UK Trent Multicentre Ethics Committee and participating centres obtained local ethical approval; written informed consent was required from parents of all patients entered onto the study. Patients under 1 year of age at diagnosis, receiving etoposide and carboplatin as part of their standard clinical treatment, were eligible. A total of 19 patients were studied at 6 different Children's Cancer and Leukaemia Group (CCLG) centres. All patients were required to have a double lumen central venous catheter in place to participate in this pharmacokinetic study.

Etoposide [5 mg/kg/day; 2 h intravenous infusion] was co-administered on each of 3 days of treatment with carboplatin [6.6 mg/kg/day; 1 h intravenous infusion] as part of the standard treatment regimen that each patient was receiving, as defined by the INES treatment protocol. Toxicity was assessed by the National Cancer Institute Common Toxicity Criteria (CTC), version 2.0, following etoposide and carboplatin treatment.

Blood sampling and analysis

Blood samples for measurement of etoposide concentrations were obtained from a central line prior to the beginning of infusion and at 1, 2 and 4 h after the start of drug

administration. Samples for measurement of carboplatin concentrations were taken prior to infusion and at 0.5, 1 and 2 h after the start of administration. All samples were taken from a different lumen to that used for drug administration. The actual times that samples were taken were recorded on each day of treatment for all patients and these accurate sampling times were used for pharmacokinetic analysis. Plasma was immediately separated from whole blood samples (2 ml) by centrifugation (1,200*g*, 4°C, 10 min) and 1 ml was then removed and placed in an Amicon Centrifree micropartition unit with a 30,000 MW cut-off (Millipore, Edinburgh, UK). This plasma sample was centrifuged (1,500*g*, 4°C, 15 min) to obtain plasma ultrafiltrate for determination of free carboplatin levels. Plasma samples were obtained on each of three consecutive days of treatment for both drugs and combined samples were taken whenever possible. For carboplatin, samples were obtained from a total of 19 patients on days 1 and 2 of treatment and from 13 of these patients on day 3 of treatment. For etoposide, samples were obtained from a total of 11 patients on day 1 of treatment, 10 of these patients on day 2 and from 6 patients on day 3. Blood samples were collected in heparinised tubes and centrifuged at 1,200*g* for 10 min at 4°C. Plasma was separated and frozen at −20°C, prior to analysis. Samples were sent by overnight courier, on dry ice and in an insulated container, to the Northern Institute for Cancer Research, Newcastle University, and were stored at −20°C prior to analysis. All samples were received in Newcastle within 24 h of being sent by the clinical centre and it was confirmed that they were maintained in a frozen state upon arrival in the laboratory.

Platinum sample analyses were carried out by flameless atomic absorption spectrophotometry (AAS) using a Perkin-Elmer AAnalyst 600 graphite furnace spectrometer (Perkin-Elmer Ltd, Beaconsfield, UK). Free or unbound platinum levels were determined in plasma ultrafiltrates as previously described [8]. All samples were analysed in duplicate and values expressed as the average of these measurements. Duplicate values were within 15% in all cases. Intra- and inter-assay coefficients of variation for a quality assurance sample had to be <10% for an assay to be valid. The limit of detection for the assay was 0.10 μg/ml.

Etoposide levels were determined using an API 2000 LC/MS/MS with analyst software (Applied Biosystems, CA, USA) following extraction from plasma samples. Briefly, plasma samples (100 μl) were extracted with ethyl acetate (1 ml), evaporated to dryness under nitrogen at 37°C and reconstituted in 100 μl mobile phase (acetonitrile:0.1% acetic acid pH 4.7; 50:50) prior to LC/MS/MS analysis. Reconstituted samples were injected onto a Genesis C_{18} 4 μm, 4 mm × 100 mm analytical column using a Series 200 autosampler (Perkin-Elmer, Buckinghamshire, UK) and eluted under isocratic conditions with mobile phase at a flow rate of 0.2 ml/min. Etoposide concentrations were quantified using a standard curve of 0.20–10.0 μg/ml. Calibration plots were linear over the concentration range studied and the assay had a limit of quantitation of 0.20 μg/ml. Intra-assay and inter-assay coefficients of variation were <10%.

Pharmacokinetic analysis

The development of population pharmacokinetic models for carboplatin and etoposide was undertaken using non-linear mixed effects modelling implemented as part of the NONMEM version VI, level 1.1 software [9]. The first-order conditional estimation method with η/ε interaction was used to obtain parameter estimates together with either ADVAN1 with the TRANS2 reparameterisation, for one-compartment models, or ADVAN3 and TRANS4 for two-compartment models.

The same basic modelling strategy was used for both etoposide and carboplatin analysis. Firstly, an appropriate structural model was obtained and then covariates were added to this basic model to establish any relationship with population parameters. A composite error model was used initially to describe the intra-subject variation, however, a proportional error model was found to be sufficient for both drugs. An additive error model, on the log scale, was used to model inter-individual variability (IIV) in pharmacokinetic parameters. Data obtained from all 3 days of treatment were modelled simultaneously allowing estimation of both IIV and inter-occasion variability (IOV). The model for each pharmacokinetic parameter incorporating IOV was as follows:

$$\log_e \theta = \log_e \tilde{\theta} + \eta + \mathrm{occ}_1 \cdot \eta_1 + \mathrm{occ}_2 \cdot \eta_2 + \mathrm{occ}_3 \cdot \eta_3$$

where $\tilde{\theta}$ is the typical value of pharmacokinetic parameter θ in the population (e.g. population mean); η is a random effect accounting for IIV; η_1, η_2 and η_3 are random effects to account for IOV and occ_n are indicator variables taking the value 1 for the nth day of treatment and 0 otherwise. IOV was considered for clearance (Cl) and central volume ($V1$) and it was assumed that IOV was the same for each day of treatment. This was implemented in NONMEM using the SAME option for the OMEGA statement.

Covariates were added in a linear fashion to the model for the logarithm of the pharmacokinetic parameter. Covariates investigated were BW, SA, age at diagnosis, sex and disease stage. The logarithmic transformation of BW, SA and age was used in the modelling process. The model incorporating a covariate without IOV was as follows:

$$\log_e \theta = \log_e \tilde{\theta} + \beta \cdot \mathrm{COV} + \eta$$

where β is the regression coefficient and COV is the covariate to be included in the model.

Choices concerning model structure and covariate selection were based upon the reduction in NONMEM objective function value (OFV), together with examination of residual plots.

The OFV was used to compare the fit of full and reduced (nested) models with p and q parameters, respectively. The difference in OFV for two such nested models has an approximate asymptotic χ^2-distribution with $(p - q)$ degrees of freedom (df). For two nested models differing by a single parameter, if the difference in OFV was larger than 3.84 (the 95th percentile of the χ^2_1 distribution) then the fuller model was deemed to provide a statistically significantly better fit to the data. Empirical Bayes estimates of pharmacokinetic parameters were obtained from the final population model.

Statistical analysis

The unpaired t test was used to determine differences between carboplatin Cl values normalized to BW observed in infants <12 kg in the current study and data obtained from older children (>12 kg) receiving carboplatin in a previously published study [10]. The logarithm of Cl/kg was used in this analysis.

Results

Patient characteristics and treatment

Nineteen infants receiving carboplatin and etoposide were entered onto the study between January, 2001 and April, 2006. The study population had a median age of 10 months at time of treatment (range 2.5–14 months) and included ten female and nine male patients. Patient characteristics including age, sex and disease stage are given in Table 1. Of the 19 patients studied, 16 received the standard carboplatin dose of 6.6 mg/kg, two received a reduced dose of 5 mg/kg and one patient received a reduced dose of 3.75 mg/kg. All 11 patients studied following etoposide treatment received the protocol-defined dose of 5 mg/kg/day.

Pharmacokinetics

Carboplatin

Plasma samples were obtained from 19 infant patients receiving carboplatin on days 1 and 2 of treatment and 13 of these patients on day 3, with an average of 4 samples being obtained per patient on each study day. A total of 75 and 74 plasma carboplatin concentrations were obtained on days 1 and 2 of treatment, respectively, with an additional 52 samples collected on day 3 of chemotherapy. All carboplatin plasma concentrations were above the limit of quantitation for the assay. Median peak plasma concentrations of unbound carboplatin (C_{max}) were 12.3 μg/ml (range 6.7–17.0 μg/ml), 11.4 μg/ml (range 7.8–15.7 μg/ml) and 13.4 μg/ml (range 7.7–19.0 μg/ml) on treatment days 1, 2 and 3, respectively.

Initial modelling highlighted two data points, one each from patients 9 and 16, that had unusually high residuals. Both of these samples were taken mid-infusion, suggesting possible contamination of the line. These data points were excluded from subsequent analyses. The structural model chosen was a two compartment model parameterised as Cl, $V1$, Q and $V2$, with random effects included for Cl, $V1$ and $V2$. No evidence of IIV in Q, or IOV in Cl or $V1$ was apparent; addition of a random effect for Q and/or random effects to allow for IOV resulted in a decrease in OFV of <1.

Covariates were added to this base model. The addition of BW or SA resulted in a large reduction in OFV. As BW and SA were highly correlated (correlation coefficient = 0.99), BW alone was used for any further analysis. Incorporating $\log_e$ BW into the model for all four population pharmacokinetic parameters and further assuming the regression coefficient to be the same for each of the parameters (Cl, $V1$, Q and $V2$) led to a reduction in OFV of 31 (1 df, $P < 0.001$). Allowing the regression coefficients to differ between pharmacokinetic parameters led to a negligible increase in OFV. The estimate of the regression coefficient for the final model was 1.01 and could be set equal to 1 with no increase in OFV. The variance/covariance matrix for this model included just one covariance term between Cl and $V1$. The removal of this covariance produced an increase of 5 in the OFV (1 df, $P = 0.03$) and the addition of further terms had negligible effect on the OFV. There was no effect on Cl of age, sex or disease stage after including BW into the covariate model.

The typical values for the pharmacokinetic parameters obtained from the final population model were Cl 22.6 ml/min; $V1$ 1.23 l; Q 67.2 ml/min and $V2$ 2.2 l, for a patient weighing 8 kg. All of the pharmacokinetic parameters increased with BW and since the regression coefficient for BW, in this population, was equal to 1, a convenient way to express the results is in terms of typical values per kg BW: Cl/kg 2.82 ml/min/kg; $V1$/kg 0.15 l/kg; Q 8.4 ml/min/kg and $V2$ 0.27 l/kg. The estimates of IIV (coefficient of variation) were Cl 16%; $V1$ 56% and $V2$ 60%. This was in comparison to the base model which did not take into account BW which had the following estimates of IIV Cl 37%; $V1$ 67% and $V2$ 73%. IIV for inter-compartmental Cl could not be characterised. An example of carboplatin data obtained from patient 1 fitted using this model is shown in Fig. 1a.

Table 1 Patient characteristics

Patient	Sex	Age (months)	BW (kg)	SA (m^2)	Neuroblastoma disease stage	Carboplatin sampling	Etoposide sampling	Total number of samples
1	F	10.0	9.7	0.48	4	Y	Y	18
2	F	11.5	8.5	0.44	4	Y	Y	12
3	M	6.5	7.4	0.40	3 (unresectable)	Y	Y	20
4	F	2.5	4.8	0.29	3 (unresectable)	Y	N	12
5	F	12.5	9.8	0.49	4	Y	Y	21
6	F	11.0	9.7	0.48	4	Y	N	12
7	M	9.0	8.8	0.45	3 (unresectable)	Y	Y	17
8	F	14.0	9.4	0.47	4	Y	Y	11
9	M	10.0	8.3	0.43	2 (unresectable)	Y	N	8
10	M	12.0	9.6	0.47	4	Y	Y	20
11	F	4.0	5.1	0.28	4	Y	N	12
12	F	6.0	6.9	0.38	4	Y	Y	18
13	M	13.0	10.3	0.50	4	Y	Y	18
14	M	7.0	8.7	0.45	4	Y	Y	12
15	M	2.5	5.4	0.31	2 (unresectable)	Y	Y	12
16	M	9.0	11.0	0.53	4	Y	N	12
17	M	12.0	8.3	0.44	3 (unresectable)	Y	N	12
18	F	4.0	4.9	0.30	3 (unresectable)	Y	N	12
19	F	12.0	8.3	0.44	3 (unresectable)	Y	N	8

BW body weight, *SA* surface area, *F* female, *M* male, *Y* yes, *N* no

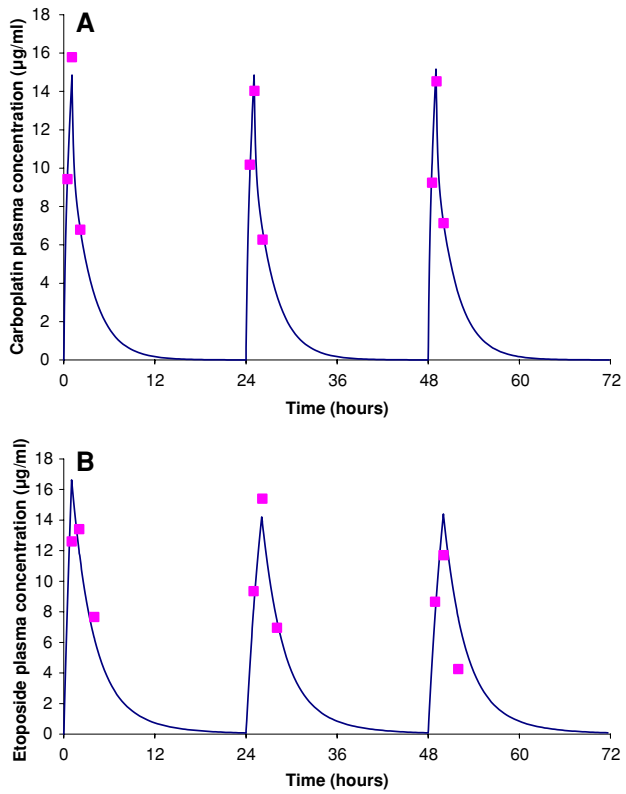

Fig. 1 Pharmacokinetic data for carboplatin (**a**) and etoposide (**b**) obtained from patient 1 fitted using a non-linear mixed effects model

Empirical Bayes estimates of carboplatin pharmacokinetic parameters obtained from the non-linear mixed effects model analysis are shown in Table 2. Estimates of Cl ranged from 12.8 to 33.6 ml/min, with a median value of 24.8 ml/min (Fig. 2a), and AUC values of 1.4–3.1 mg/ml.min were achieved on each day of treatment, i.e. 4.2–9.3 mg/ml.min cumulative AUC values. The half-life ($t_{1/2}$) of carboplatin had a median value of 2.0 h, with a range of 0.9–6.6 h and C_{max} varied between 7.8 and 17.0 µg/ml (median 12.9 µg/ml).

The relationship between patient BW and carboplatin Cl normalized to BW in children <1 year of age recruited to the current study, compared to data from older children receiving carboplatin (doses of 100–900 mg/day) obtained from a previously published study [10] was investigated. This additional study included two patients with BW <12 kg and ten patients with BW >12 kg. While a good correlation was observed in general between carboplatin Cl and BW when including the older patient group, Cl values normalized to BW were significantly higher in patients <12 kg than in children >12 kg (Fig. 3). Children <12 kg had on average a 1.14 times larger Cl/kg than children >12 kg (95% CI 1.00, 1.29), with mean ± SD values of 2.9 ± 0.4 and 2.5 ± 0.4 ml/min/kg, respectively ($P < 0.05$). These results suggest that Cl in an infant patient population may be higher than that predicted from data in older children.

Table 2 Non-linear mixed effects model analysis of carboplatin pharmacokinetic data following carboplatin treatment [6.6 mg/kg/day] in children <1 year of age ($n = 19$)

Patient	Carboplatin dose [mg]	C_{max} [μg/ml]	$t_{1/2}$ [h]	AUC [mg/ml.min]	Cl [ml/min]	Cl [ml/min/kg]
1	64	15.8	1.9	2.3	27.9	2.87
2	56	9.4	4.1	2.7	21.0	2.47
3	37	17.0	1.0	1.9	19.8	2.68
4	18	7.8	1.9	1.4	12.8	2.66
5	64	9.6	3.2	1.9	33.6	3.43
6	64	13.9	1.6	2.1	30.7	3.16
7	57	17.0	0.9	1.9	30.8	3.50
8	62	8.8	6.6	2.8	21.9	2.33
9	55	14.6	1.4	1.9	29.3	3.53
10	63	12.3	2.9	3.1	20.2	2.11
11	34	14.2	2.0	2.3	15.1	2.95
12	46	12.9	2.5	2.8	16.7	2.42
13	70	16.1	1.7	2.4	28.6	2.78
14	55	14.4	1.5	2.0	27.0	3.10
15	36	12.2	2.9	2.8	12.9	2.39
16	70	9.8	2.5	2.2	32.4	2.95
17	54	14.0	1.6	2.2	24.8	2.98
18	22	8.0	2.6	1.7	13.0	2.66
19	56	12.9	2.6	2.1	26.2	3.15
Mean		12.7	2.4	2.2	23.4	2.85
SD		3.0	1.2	0.44	7.0	0.41
Median		12.9	2.0	2.2	24.8	2.87
Range		7.8–17.0	0.9–6.6	1.4–3.1	12.8–33.6	2.11–3.53

Patients 3 and 18 received a reduced carboplatin dose of 5.0 mg/kg/day; patient 4 received a reduced carboplatin dose of 3.75 mg/kg/day

C_{max} peak plasma concentration, *AUC* area under the plasma concentration–time curve (daily AUC values provided), *Cl* clearance, *SD* standard deviation

Etoposide

A total of 55 plasma etoposide concentrations were obtained from 11 patients on day 1 of treatment, 43 concentrations from 10 patients on day 2 and a further 24 samples from 6 of these patients on day 3. All etoposide plasma concentrations were above the limit of quantitation for the assay. The structural model chosen for etoposide analysis was a two compartment model parameterised as Cl, $V1$, Q and $V2$, with random effects included for Cl and $V1$; a covariance term was also included to allow for correlation between the two parameters. There was no evidence of IIV in Q or $V2$. Inclusion of random effects to account for IOV in Cl or $V1$ resulted in negligible change in OFV with the estimates of variance for these terms tending towards 0.

The addition of BW or SA to this base model resulted in a small reduction in OFV. BW alone was chosen for further analysis due to the very large correlation between BW and SA. Fixing the regression coefficients to be the same for all four pharmacokinetic parameters only reduced the OFV by 1.5 (1 *df*, $P = 0.22$). A larger reduction of 9.6 (4 *df*, $P = 0.05$) was obtained by allowing the regression parameters to vary independently, however, this resulted in a negative slope for $\log_e Q$ which is not plausible physiologically. A pragmatic approach was adopted by setting the regression coefficients for Cl and Q to be the same and the regression coefficients for $V1$ and $V2$ to be the same. This reduced the OFV by 5.3 (2 *df*, $P = 0.07$). The estimates of the regression coefficients for the final model were 1.5 for Cl and Q, and 0.76 for $V1$ and $V2$. There was no effect on Cl of age, sex or disease stage after including BW into the covariate model.

Typical values for the pharmacokinetic parameters obtained from the final population model were Cl 6.3 ml/min; $V1$ 1.3 l; Q 1.08 ml/min and $V2$ 0.35 l, for a patient weighing 8 kg. The covariate model for Cl (ml/min) may be written as follows:

$$\mathrm{Cl} = 6.3 \left(\frac{\mathrm{BW}}{8}\right)^{1.5}.$$

The estimates of IIV (coefficient of variation) were Cl 35% and $V1$ 40%, compared to the base model not taking into account BW which had estimates of: Cl 47% and $V1$ 44%.

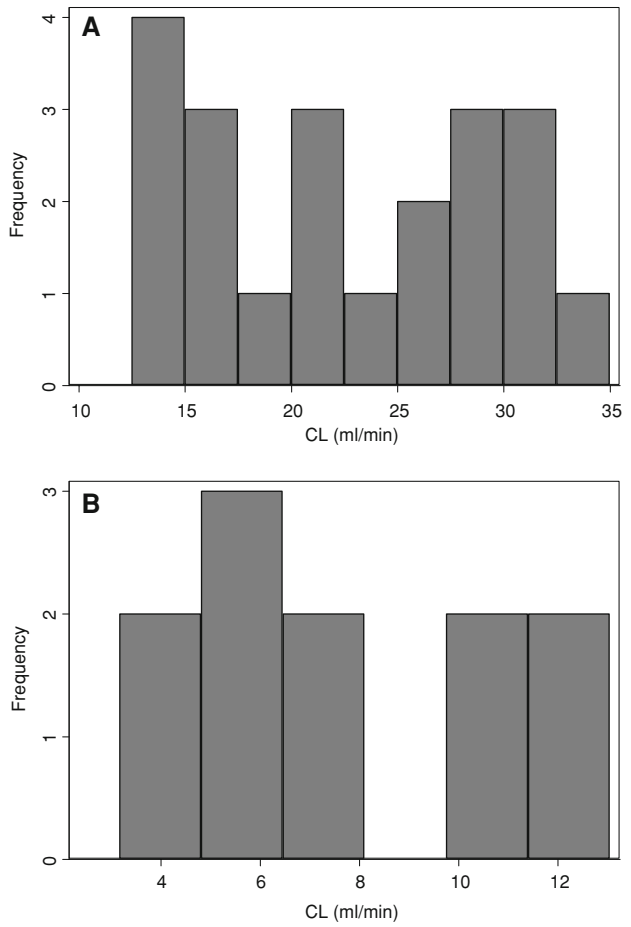

Fig. 2 Histograms of clearance for **a** carboplatin ($n = 19$) and **b** etoposide ($n = 11$) in children <1 year of age determined using a non-linear mixed effects model

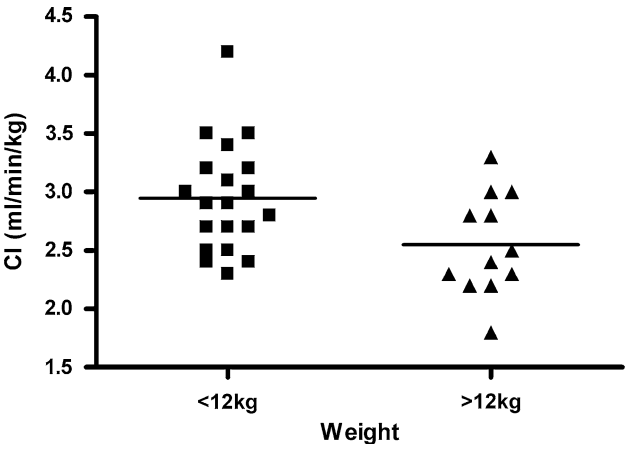

Fig. 3 Carboplatin clearance normalized to body weight in infant patients (<12 kg) as compared to data from older children (>12 kg) obtained in a previously published study [10]

An example of etoposide data obtained from patient 1 fitted using this non-linear mixed effects model is shown in Fig. 1b and estimates of pharmacokinetic parameters are shown in Table 3 for all eleven patients. The half-life ($t_{1/2}$) of etoposide on day 1 had a median value of 4.6 h, with a range of 4.1–6.6 h. The median AUC was 7.1 mg/ml.min [range 3.4–11.0 mg/ml.min], C_{max} varied between 12.5 and 41.2 μg/ml (median 25.1 μg/ml) and estimates of Cl ranged from 3.2 to 13.0 ml/min (median 6.6 ml/min).

Toxicity and response

Toxicity data were available on the relevant cycle of treatment for all 19 patients studied. CTC grade 3 or 4 toxicity was observed in five patients (26%) and included grade 3 and 4 haematological toxicity (neutropaenia, thrombocytopenia, haemoglobinaemia and leucocytopaenia), infection and elevated concentrations of alanine transaminase (ALT). No relationship was observed between incidence of toxicity and AUC values of carboplatin or etoposide or any other pharmacokinetic parameters.

Clinical response and follow-up data were available for 18 of the 19 patients studied, with a median follow-up of 2.9 years (range 0.3–6 years). Complete remission observed in eight patients (44%) following treatment which included carboplatin and etoposide. Follow-up data available for these 18 patients indicated a complete response rate of 44% (8/18 patients), with minimal or stable disease present in an additional eight patients (44%), with two patients (11%) having died following disease recurrence. There was no relationship observed between either remission data or current patient status and carboplatin or etoposide pharmacokinetic parameters.

Discussion

There have been only a limited number of studies on the pharmacokinetics of anticancer drugs in infants and very young children [11–13]. Such studies are essential to understand the potential impact of dose reductions prescribed in current infant dosing protocols. It is important to compare the degree of inter-patient variation in pharmacokinetics following infant dosing regimens with that previously observed in older children.

The current study describes the pharmacokinetics of the commonly used anticancer drugs carboplatin and etoposide in infants diagnosed with neuroblastoma at <1 year of age. A total of 19 patients were studied over a period of 5 years in six UK centres. Due to concerns over the possible clinical implications of taking multiple blood samples from very young patients, several approaches were used to minimize any risk. A limited sampling pharmacokinetic approach was used to determine the pharmacokinetics of carboplatin and etoposide in these patients, with only three blood samples taken on each of the 3 days of treatment.

Table 3 Non-linear mixed effects model analysis of etoposide pharmacokinetic data following etoposide treatment [5.0 mg/kg/day] in children <1 year of age ($n = 11$)

Patient	Etoposide dose [mg]	C_{max} [μg/ml]	$t_{1/2}$ [h]	AUC [mg/ml.min]	Cl [ml/min]	Cl [ml/min/kg]
1	50	15.4	4.1	3.8	13.0	1.35
2	42	13.0	4.4	3.4	12.3	1.45
3	37	17.1	5.1	5.3	7.0	0.95
5	49	12.5	4.2	4.6	10.6	1.08
7	43	39.0	4.6	7.1	6.0	0.69
8	47	16.4	4.3	4.7	10.1	1.07
10	48	30.3	5.1	8.7	5.5	0.57
12	35	36.4	6.1	11.0	3.2	0.46
13	52	41.2	4.4	7.9	6.6	0.64
14	44	25.1	5.2	8.1	5.4	0.62
15	27	27.6	6.6	7.9	3.4	0.64
Mean		24.9	4.9	6.6	7.6	0.87
SD		10.7	0.83	2.4	3.4	0.33
Median		25.1	4.6	7.1	6.6	0.69
Range		12.5–41.2	4.1–6.6	3.4–11.0	3.2–13.0	0.46–1.45

C_{max} peak plasma concentration, *AUC* area under the plasma concentration–time curve (daily AUC values provided), *Cl* clearance, *SD* standard deviation

The sampling times selected were based on obtaining plasma levels for both drugs at the mid-point of infusion, upon completion of infusion and at 1–2 h post-end of infusion. For carboplatin these time points have previously been utilised for a limited sampling Bayesian approach in older children [10]. However, it should be noted that the sampling times used were largely selected based on what was felt to be practicable in this patient population and clinical setting. Due to the limited number of samples obtained in this study, the development of a non-linear mixed effects model was felt to be the most appropriate approach to data analysis. In addition to the use of a limited sampling approach, in order to minimize the volume of blood required for measuring etoposide levels in plasma, a novel liquid chromatography mass spectroscopy (LC–MS) method was developed to allow the analysis of etoposide from a minimum volume of plasma.

Carboplatin data analysis using a non-linear mixed effects model, provided estimates of AUC values ranging from 1.4 to 3.1 mg/ml.min on a single day of treatment, with cumulative AUC values over the 3 days of treatment ranging between 4.2 and 9.3 mg/ml.min. The overall carboplatin AUC values achieved in many infant patients studied are lower than those defined in the majority of conventional carboplatin dosing regimens in older children, with AUC values above 7 mg/ml.min commonly targeted in previously untreated patients [14]. While it could be argued that infant patients are more likely to be susceptible to the side effects of chemotherapy, thereby warranting lower target AUCs, the incidence of grade 3/4 toxicity of approximately 26% observed in the current study does not convincingly support this theory. A comparison of carboplatin Cl values normalized to BW indicated a significant difference in values between infant patients and older children (>12 kg), with higher normalized Cl values seen in the younger patients. In this respect it may be that while Cl values are comparable in infants and older children in general terms, prediction of absolute carboplatin Cl values in individual patients may be more difficult in very young patients. This is supported by a recent publication reporting marked changes in carboplatin Cl values with age in a preterm infant with retinoblastoma and would indicate that infant patients may benefit from pharmacokinetically guided carboplatin treatment [15]. Interestingly, whereas this previously published case report showed clear increases in carboplatin Cl over a relatively short time period of 2 months, the current data do not indicate a significant change in systemic Cl over the first year of life. While this does not entirely fit with the concept of maturation of renal function that occurs during infant development, it may be a trend that is difficult to identify in the small patient population studied. As was seen convincingly in the preterm infant case report study, such a trend may well be more appropriately investigated by obtaining estimates of pharmacokinetic parameters over several courses of treatment in individual patients.

Etoposide pharmacokinetic data obtained in the current study using a non-linear mixed effects model indicated Cl values of 3.2–13.0 ml/min [8.4–28.0 ml/min/m^2], AUC values of 3.4–11.0 mg/ml.min and half-lives of 4.1–6.6 h.

These values are comparable to those previously reported from several published studies in older children and are in agreement with previous findings that question the utility of modified dosing in infants [12, 16, 17]. For example, despite differences in the length of etoposide infusion times between the current study and a study previously published by Boos et al., very similar estimates of Cl were obtained in a comparable patient population [0.9 ± 0.3 vs. 0.8 ± 0.3 ml/min/kg]. The study by Boos et al. represents the only previous study to have published comprehensive etoposide pharmacokinetic data in an infant patient population and strongly suggests that special dose-calculation guidelines for infants are not justified by age-dependent pharmacokinetics or tolerance. A more recent study which included a limited number of infants in a larger paediatric leukaemia patient cohort also reported comparable etoposide pharmacokinetics, with a median Cl of 17.1 ml/min/m^2 or 0.9 ml/min/kg [17]. Despite the limited number of patients, these data again indicate that distinct dose-calculation guidelines are not required for infants above 3 months of age.

When considering the pharmacokinetic data obtained in this study, in addition to drawing comparisons with previously obtained paediatric data, it is clearly important to consider the clinical responses observed in this patient group. Of the patients studied, complete responses were obtained in 44% of infant patients and partial responses or stable disease achieved in an additional 44% of patients. Indeed, clinical data obtained from the INES on which these patients were being treated, indicates very high event-free survival rates of >85% in all patients with non-MYCN amplified tumours, suggesting that the chemotherapy regimens utilized are highly effective. However, it is possible that for malignancies associated with lower response rates in infants and very young children, more rational and moderate dose modification in infants may be beneficial. In this scenario, dose adjustments could be determined by the monitoring of toxicity in addition to adaptive dosing based on pharmacokinetic profiles in individual patients.

The current study demonstrates the feasibility of generating informative pharmacokinetic data in an infant patient population. Important considerations when carrying out such studies include the requirement for sensitive analytical techniques to allow quantification of drug levels from a minimum sample volume and the use of limited sampling approaches. With the recent instigation of the European Paediatric Regulation and associated provision of funding for studies into off-patent medicinal products through the EU Framework programmes, there is real potential for further data to be acquired to guide the future use of anticancer drugs and other medicinal products in infants and young children.

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Conflict of interest statement None.

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