EUROPEAN JOURNAL OF CANCER 47 (2011) 1556–1563

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Pharmacokinetics of cyclophosphamide and its metabolites in paediatric patients receiving high-dose myeloablative therapy

Girish Chinnaswamy [a], *Julie Errington* [a], *Annabel Foot* [b], *Alan V. Boddy* [a], *Gareth J. Veal* [a], *Michael Cole* [a,*]

[a] *Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne NE2 4HH, UK*
[b] *Bristol Royal Hospital for Children, Bristol BS2 8BJ, UK*

ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 16 February 2011
Accepted 8 March 2011
Available online 7 April 2011

Keywords:
Cyclophosphamide
Myeloablative
Children
NONMEM
Pharmacokinetic

ABSTRACT

Introduction: In order to better understand the impact of high-dose on the pharmacokinetics and metabolism of cyclophosphamide, a pharmacological study was performed in children with malignant mesenchymal tumours with metastatic disease.

Methods: Patients received four courses of chemotherapy including two courses of cyclophosphamide. Plasma concentrations of cyclophosphamide and the metabolites 4-ketocyclophosphamide, dechloroethylcyclophosphamide and carboxyphosphamide were determined on days 1, 2 and 3 of each course. A population pharmacokinetic model for cyclophosphamide was developed using non-linear mixed effects modelling and metabolite AUC values compared between days and courses.

Results: Data were available on 21 cyclophosphamide courses from 15 patients. A one compartment model, incorporating a term in surface area for both *CL* and *V*, best described cyclophosphamide pharmacokinetics. Typical *CL* and *V* on day 1 of treatment for a patient with a SA of 1.4 m^2 were 4.3 L/h and 28.5 L, respectively. On days 2 and 3 *CL* increased by 88% (95% CI, 72–105%) and 125% (95% CI, 108–145%) over day 1 levels; *V* increased by 14% (95% CI, 5–23%) on days 2 and 3. *V* tended to be larger for males than similarly sized females but no effect of age was found upon *CL* or *V*. Significant increases in metabolite AUCs were observed on days 2 and 3 compared to day 1 and a significant increase in CXCP AUC from course 1 to course 3.

Conclusion: Administration of high-dose cyclophosphamide over several days results in an increase in metabolism, possibly by induction of the activation pathway. This induction is effectively reversed following a four week period between cyclophosphamide doses. The degree of intersubject variation in cyclophosphamide elimination is largely accounted for by body surface area and is less than previously reported.

1. Introduction

Cyclophosphamide is an alkylating agent used in the treatment of paediatric cancers including leukaemias, lymphomas,[1] rhabdomyosarcoma,[2] Ewing's sarcoma,[3] neuroblastoma[4], and brain tumours.[5] Higher doses are used for myeloablative conditioning prior to bone marrow transplantation.[6] The concept of high-dose cyclophosphamide relies

* *Corresponding author:* Address: Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK. Tel.: +44 1912464348; fax: +44 1912464301.
E-mail address: michael.cole@newcastle.ac.uk (M. Cole).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.03.008

on a steep dose–response curve for antitumour effect. Given the wide range of cyclophosphamide doses, differences in pharmacokinetics and metabolism are likely to occur. This is further complicated by autoinduction on prolonged or repeated administration and saturation of metabolism at higher doses. The elimination rate of cyclophosphamide correlates inversely with formation of the active 4-hydroxy metabolite.[7] In non-Hodgkins lymphoma, patients with recurrent disease had a lower cyclophosphamide clearance than those in sustained remission.[8] Conversely, patients with recurrent disease had higher plasma concentrations of the inactive dechlorethyl and carboxy metabolites.[8] Similar observations linking higher cyclophosphamide clearance with greater pharmacological activity have been made in patients with breast cancer.[9]

Rhabdomyosarcoma is the most common soft tissue sarcoma (STS) in children.[10] Poor risk groups, such as patients with metastatic disease at presentation, have 5-year survival rates varying between 20–30%.[11–13] In poor risk STS patients, high dose chemotherapy with stem cell rescue, using various single and multiagent chemotherapy schedules with or without radiotherapy, have had limited success.[14–16] The MMT98 study aimed to investigate the benefit of sequential high dose chemotherapy schedules[17] in this poor risk group of childhood cancer patients.[18]

In order to better understand the impact of high-dose on pharmacokinetics and metabolism of cyclophosphamide, a pharmacological study was performed in children treated on the MMT98 protocol. The approach taken incorporated a population pharmacokinetic modelling approach to investigate the sources of variability in plasma concentrations of cyclophosphamide and its metabolites amongst the defined target patient population. Such an approach allows the identification of key factors that influence dose–concentration relationships and which may therefore impact clinically in terms of response or incidence of toxicity in patients receiving high dose cyclophosphamide.

2. Methods

2.1. Patients and treatment

The MMT98 study addressed the treatment of metastatic STS in children and young adults. Fifteen poor risk patients (age>10 years and/or with bone/bone marrow involvement) were recruited (median age 13.3 years, range 5.4–21 years). Phase one of treatment was a phase II window study.[19] Phase two consisted of early high dose sequential monotherapy, followed by stem cell rescue, patients also received maximal local therapy. Phase three consisted of 3-weekly cycles of vincristine, cyclophosphamide, and actinomycin D given for a total of 9 cycles.

Patients with pathologically-confirmed rhabdomyosarcoma or other malignant mesenchymal tumours with evidence of metastatic disease being treated under the MMT 98 study as poor risk patients were entered into the pharmacology study. Patients received four courses of chemotherapy at intervals of 14 days without awaiting blood count recovery.

Course 1: Cyclophosphamide (2 g/m^2/day), days 1, 2, and 3 (1 h infusion),
Course 2: Etoposide (800 mg/m^2/day), days 15, 16, and 17 (24 h infusion),
Course 3: Cyclophosphamide (2 g/m^2/day), days 29, 30, and 31 (1 h infusion),
Course 4: Carboplatin (AUC 4 mg/ml min/day), days 44–48 (1 h infusion).

Written informed consent was obtained prior to the study from each patient or legal guardian. The study received approvals by national and local ethics committees.

2.2. Blood sampling and analysis

Blood samples for pharmacokinetic analysis were obtained from a central line on day 1 of courses 1 and 3 at 0, 0.5, 1, 2, 4, 6, and 8 h after the start of infusion (study days 1 and 29). Samples were also taken at 0, 1, 4, and 8 hours on days 2 and 3 of each course. Blood samples (3 ml) were collected in heparinised tubes and centrifuged at 2000*g* at 4 °C. Plasma was separated and stored at −20 °C prior to analysis. Concentrations of cyclophosphamide and the inactive metabolites, 4-ketocyclophosphamide (KetoCP), dechloroethylcyclophosphamide (DCCP), and carboxyphosphamide (CXCP) were measured in plasma using a validated LCMS method. Cyclophosphamide was obtained from Sigma Chemical co, Poole Dorset. The inactive metabolites and the internal standard deuterated cyclophosphamide (D_4CP) were obtained from IIT, University of Bielefeld, Germany. In Eppendorf tubes, 300 μl of each plasma sample was mixed with 1.65% acetic acid (210 μl), distilled water (40 μl) and internal standard (deuterated cyclophosphamide: 50 μl of 5 μg/ml) and vortex mixed. The extraction process was carried out on an automated Gilson ASPEC XL4 system using the ASPEC 735 sampler software. C_{18} SPE columns (supplied by Kinesis, UK) were preconditioned with 3 ml of acetonitrile, followed by 3 ml of 0.5% acetic acid. Plasma samples were loaded and the SPE columns were washed with 0.5% acetic acid. The compounds of interest were then eluted with 3 ml of acetonitrile and eluates evaporated to dryness at 37 °C. The residues were reconstituted in 200 μl of mobile phase. Reconstituted samples were analysed using an Applied Biosystems/MDS SCIEX/3200 QTRAP LC/MS system and Analyst software version 1.4.2 (Applied Biosystems, Foster city, California, USA). Chromatographic separation of the compounds of interest was achieved using a Phenomenex Luna C-8(2) 50 × 2 mm (3μ) column with a flow rate of 300 μl/min (50:50 0.1% formic acid: methanol). The electrospray source was operated in the positive ion mode. The retention times and mass transitions of cyclophosphamide, metabolites and the internal standard were: cyclophosphamide 2.4 min, 261/140; CXCP 2.0 min, 293/221; DCCP 1.7 min, 199/171; KetoCP 1.9 min 275/142; D_4-cyclophosphamide 2.4 min 267/145. Simultaneous standard curves for cyclophosphamide and metabolite peak areas, corrected for that of the internal standard were analysed. QC samples for each analyte were included in each assay. Back calculated concentrations of standards and QCs were within 85–115% of expected values for the run to be valid. Standard curves were linear between

0.5–10 μg/ml for cyclophosphamide and 0.05–1 μg/ml for CXCP, DCCP and KetoCP with r^2 values $\geqslant$0.99. Samples containing concentrations of cyclophosphamide or metabolites above the linear range were diluted with blank plasma.

2.3. *Pharmacokinetic analysis*

A population pharmacokinetic model for cyclophosphamide was developed using non-linear mixed effects modelling (NONMEM version VI, level 1.1). The first order conditional estimation method (FOCE) with η/ε interaction was used, together with ADVAN1 and the TRANS2 reparameterisation. Change in NONMEM objective function value (OFV), and examination of residual plots guided selection of model structure and covariates. The difference in OFV (between two nested models differing by a single parameter) was required to be considerably larger than 3.84, in addition to improvements in residual plots, before accepting the fuller model.[20] An appropriate structural model was identified before examining the influence of covariates. A composite error model was most appropriate to describe within-subject error. An additive error model, on the logarithmic scale, was used for inter-individual variability (IIV) in pharmacokinetic parameters. Additional error terms were included to account for inter-occasion variation (IOV), assumed to be the same for each course of treatment. Covariates (body weight, surface area, age at diagnosis, and sex) were added in a linear fashion to the model for the logarithm of the pharmacokinetic parameter. Logarithmic transformations of body weight and surface area were used. Population models were fitted to data obtained from days 1, 2, and 3 of courses 1 and 3. Empirical Bayes estimates of pharmacokinetic parameters were obtained from the final population model.

Plasma concentrations of the inactive metabolites CXCP, DCCP and KetoCP were determined on days 1, 2, and 3 of the cyclophosphamide course. The metabolite AUCs achieved were estimated by non-compartmental analysis from time 0–8 h. Logarithmically transformed AUCs were analysed for differences between day of treatment and between course of treatment using analysis of variance (ANOVA). Patient ID was included as a main effect to ensure course of treatment was estimated from patients who received two courses. The relationship between metabolite and parent AUC was assessed for each course and day of treatment by linear regression.

3. Results

The study ran for four years and stopped when analysis of initial data showed no benefit of high dose sequential monotherapy in high-risk STS patients.[18] Data on carboplatin pharmacokinetics obtained from this patient cohort have previously been published.[21] The results of the cyclophosphamide analysis are presented here.

Each patient received two cycles of cyclophosphamide (course 1 and course 3 of the high dose protocol). Pharmacokinetic and metabolite data were available on 21 cyclophosphamide courses obtained from 15 patients. Five patients were studied on course 1 alone, four on course 3 alone and six on both courses. Physical characteristics of the 15 patients are given in Table 1. No patient had significant renal dysfunction prior to chemotherapy, as evidenced by a normal GFR or serum creatinine. Plasma concentrations of cyclophosphamide, CXCP, DCCP and KetoCP are shown in Fig. 1 for a representative patient treated with 2 g/m^2 as a 1 h infusion (MMT98).

The best fit of the population analysis was obtained with a one compartment model characterised by clearance (*CL*) and volume of distribution (*V*). The models for *CL* and *V* included a term for surface area (*SA*). *CL* was allowed to vary randomly between individuals (IIV) and between courses (IOV) within the same individual, however it was not possible to characterise IIV and IOV for *V*. In addition, a fixed effect was included to allow for differences in *CL*, and *V*, between days of treatment on the same course. The final models were:

$$\log_e(CL) = 1.45 + 0.79 \log_e\left(\frac{SA}{1.4}\right) + 0.63\, DAY_2 + 0.81\, DAY_3$$

$$\log_e(V) = 3.35 + 1.07 \log_e\left(\frac{SA}{1.4}\right) + 0.128\,(DAY_2 + DAY_3) - 0.166\ SEX$$

CL and *V* are measured in L/h and L, respectively; DAY_2 is 1 for day 2 of the cycle, 0 otherwise (DAY_3 is similarly defined); SEX is 1 for females and 0 for males. Coefficients of variation for IIV and IOV in *CL* were 11% and 10%, respectively. Individual fits of the model are shown in Fig. 2, with population weighted residuals plotted against individual population predictions and time in Fig. 3.

Typical *CL* on day 1 of treatment for a patient with a *SA* of 1.4 m^2 was 4.3 L/h. On days 2 and 3 typical *CL* increased to 8.0 L/h and 9.6 L/h, respectively; an 88% (95% CI, 72–105%) and 125% (95% CI, 108–145%) increase over day 1 levels. For a similar 1.4 m^2 patient, typical *V* was 28.5 L on day 1 and 32.4 L on days 2 and 3 of treatment, a 14% (95% CI, 5–23%) increase. On average *V* was 18% (95% CI, 10–27%) larger for males than similarly sized females, however males tended to be older in this cohort of patients (range: males 12.0–21.0; females 5.4–16.8 years). After allowing for differences in body size and sex, there was no influence of age upon *CL* or *V*. By incorporating a fixed effect for course of treatment, *CL* was estimated to be 10% higher on course 3 than course 1 (95% CI, 1–19%). No systematic difference in *V* was observed across the courses.

Mean cyclophosphamide AUC decreased from 39.6 ± 6.0 mg/ml min (mean ± SD) on day 1 to 21.1 ± 3.2 mg/ml min on day 2 and 17.6 ± 2.7 mg/ml min on day 3. Half-life decreased from 256 ± 41 min to 129 ± 21 min comparing day 1 to day 3. The AUCs from time 0–8 h achieved for the three metabolites are shown in Table 2. For each of the metabolites there was a significant increase in AUC on days 2 and 3 compared to day 1 of treatment, with the AUC for CXCP being higher than the other metabolites (allowing for differences in molecular weight). Relative to day 1 levels, CXCP AUC increased by an average of 65% (95% CI, 46–87%) on day 2 and 56% (95% CI, 38–78%) on day 3. A similar pattern was observed for DCCP and KetoCP; the average increases were 44% and 18% for DCCP and 46% and 53% for KetoCP. There was a significant

Table 1 – Patient characteristics and empirical Bayes estimates of PK parameters on day 1 of treatment.

ID	Sex	Weight (Kg)	SA (m^2)	Age (years)	CL (L/h)	V (L)	AUC (mg/ml min)
Course 1							
101	Female	45.4	1.40	11.0	3.43	24.2	49.0
102	Male	64.3	1.75	14.6	5.81	36.3	36.1
103	Female	42.6	1.30	12.7	3.10	22.4	50.3
104	Female	24.6	0.92	8.0	2.74	15.5	40.3
105	Male	94.7	2.20	21.0	5.89	46.4	44.9
106	Female	36.3	1.20	10.6	3.63	20.6	39.7
107	Male	51.2	1.50	13.3	4.79	30.8	37.6
108	Male	25.0	0.92	12.0	3.28	18.3	33.7
109	Male	61.5	1.70	16.9	6.07	35.2	33.6
110	Female	22.1	0.85	5.4	3.42	14.2	29.8
111	Female	63.0	1.70	16.8	4.32	29.8	47.2
	Mean	48.2	1.40	12.9	4.23	26.7	40.2
	Range	(22.1, 94.7)	(0.85, 2.2)	(5.4, 21.0)	(2.74, 6.07)	(14.2, 46.4)	(29.8, 50.3)
Course 3							
102	Male	60.7	1.68	14.6	5.97	34.8	33.8
103	Female	39.4	1.30	12.7	3.25	22.4	48.0
104	Female	23.6	0.92	8.0	3.62	15.5	30.5
106	Female	36.3	1.20	10.6	3.86	20.6	37.3
107	Male	51.2	1.50	13.3	4.59	30.8	39.2
111	Female	63.8	1.70	16.8	4.80	29.8	42.5
112	Male	54.0	1.60	16.9	4.16	33.0	46.1
113	Female	30.1	1.10	8.6	3.30	18.7	36.3
114	Male	85.0	2.10	17.5	6.63	44.1	38.0
115	Female	55.5	1.60	15.0	5.07	28.0	37.9
	Mean	50.0	1.47	13.4	4.53	27.8	39.0
	Range	(23.6, 85.0)	(0.92, 2.10)	(8.0, 17.5)	(3.25, 6.63)	(15.5, 44.1)	(30.5, 48.0)

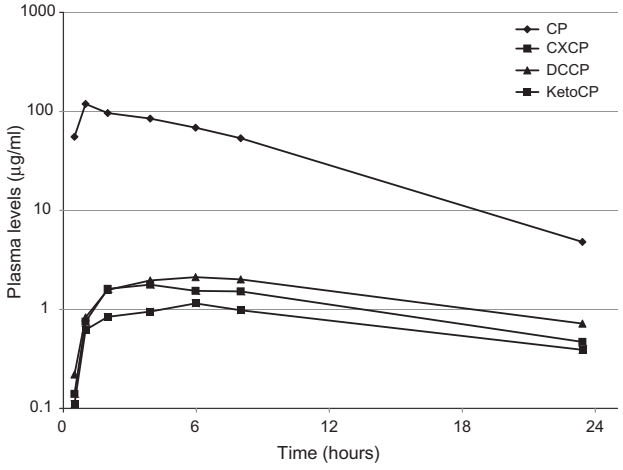

Fig. 1 – Plasma levels of cyclophosphamide and metabolites obtained from day 1 of course 1 from patient 101.

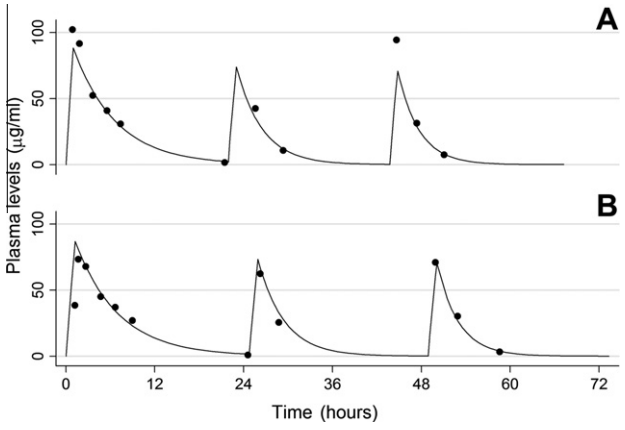

Fig. 2 – Cyclophosphamide data and population model fits (PRED, one-compartment) for patient 102; (A) course 1, (B) course 2.

increase in CXCP AUC levels from course 1 to course 3; the average increase was 16% (95% CI, 1–33%). No such difference was observed for DCCP or KetoCP.

The relationship between metabolite and parent AUC is shown in Fig. 4 for course 1 of treatment. Clearly evident is the marked increase in metabolite AUC on days 2 and 3 together with a corresponding decrease in cyclophosphamide AUC. However, for any given day and course of treatment, metabolite AUC was not statistically significantly correlated with cyclophosphamide AUC.

4. Discussion

Cyclophosphamide undergoes a complex process of both activation and inactivation in the body. Although the drug is one of the earliest chemotherapeutic agents, the complex mechanism of metabolic variations in different populations is not completely clear.[22,23] Cyclophosphamide has a steep dose–response curve and this makes it a good candidate for dose intensification or high dose therapy.[24,25] Cyclophosphamide

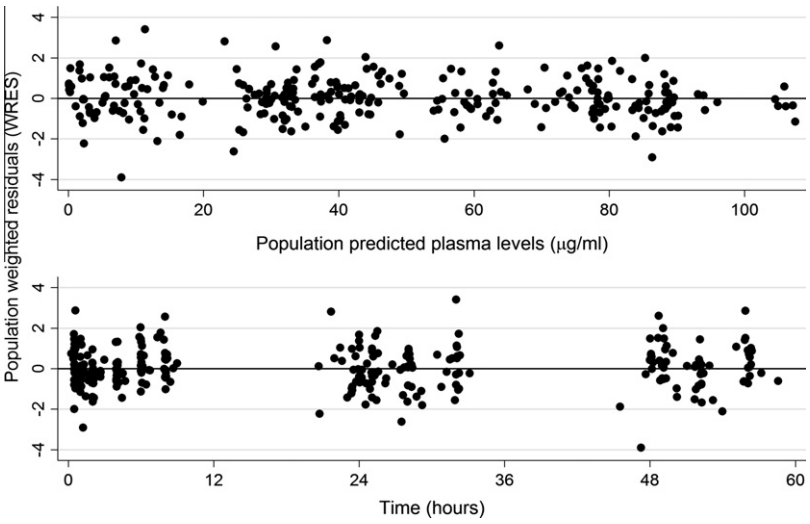

Fig. 3 – Plots of population weighted residuals (WRES) as they vary with model-predicted values (PRED) and time.

is used in high dose regimens at doses up to 6000 mg/m^2 over 4 days.[25,26] Conventional doses of cyclophosphamide are in the range of 500 to 1500 mg/m^2 administered every 3–4 weeks.

The activating metabolism of cyclophosphamide is saturable at high doses. When the dose was escalated 8-fold (500 mg/m^2 compared with 100 mg/kg over 1 h) formation of 4-hydroxycyclophosphamide was significantly reduced, whereas renal clearance and DCCP formation increased.[7,27] *In vitro* studies with liver microsomes have demonstrated saturation of 4-hydroxylation, but not side chain oxidation.[28,29] Since high concentrations of cyclophosphamide may have an unfavourable impact on drug activation, splitting the doses may be favourable. Higher doses of cyclophosphamide also lead to increased toxicity such as cardiac toxicity, haemorrhagic cystitis, water retention and hyponatraemia. There are significant variations in cyclophosphamide metabolism

Table 2 – AUC (mg/ml min), from time zero to 8 hours, of the three inactive metabolites (ND: no adequate samples available).

Patient no.	AUC_{0-8} CXCP			AUC_{0-8} DCCP			AUC_{0-8} KetoCP		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Course 1									
101	0.67	1.84	1.42	0.80	1.45	0.81	0.42	0.63	0.63
102	1.52	2.92	2.45	0.55	1.05	0.97	0.68	1.50	1.78
103	0.69	1.38	1.46	0.68	1.18	0.94	0.42	0.68	0.65
104	0.88	1.18	1.50	1.17	1.40	1.28	0.52	0.69	1.13
105	0.65	1.83	1.57	0.29	0.63	0.47	0.27	0.75	0.75
106	0.89	1.06	1.19	0.75	1.10	1.12	0.92	1.16	1.27
107	0.52	0.96	0.84	0.61	0.82	0.68	0.49	0.66	0.68
108	1.93	1.67	1.39	0.32	0.31	0.27	1.05	0.57	0.54
109	0.58	1.31	ND	0.20	0.23	ND	0.31	0.51	ND
110	1.25	1.32	1.90	0.71	0.66	0.51	0.92	0.96	0.91
111	0.71	0.98	0.99	0.53	0.79	0.45	0.40	0.53	0.43
Mean	0.94	1.50	1.47	0.60	0.87	0.75	0.58	0.79	0.88
Range	0.52, 1.93	0.96, 2.92	0.84, 2.45	0.20, 1.17	0.23, 1.45	0.27, 1.28	0.27, 1.05	0.51, 1.50	0.43, 1.78
Course 3									
102	1.27	ND	2.42	1.02	ND	1.41	0.67	ND	1.73
103	0.73	1.32	1.48	0.67	1.26	0.95	0.36	0.50	0.54
104	1.26	1.91	1.31	0.92	1.22	0.62	0.36	0.82	0.64
106	1.12	1.30	1.34	1.02	1.06	1.04	0.94	1.07	1.21
107	0.57	0.91	0.99	0.54	0.84	0.70	0.34	0.49	0.67
111	0.99	1.85	1.59	0.49	1.07	0.77	0.39	0.80	0.83
112	0.80	1.35	1.28	0.38	0.77	0.65	0.28	0.42	0.33
113	1.23	1.92	1.74	0.51	0.57	0.59	0.39	0.55	0.58
114	0.67	0.86	0.81	0.41	0.56	0.54	0.46	0.59	0.59
115	0.90	1.85	ND	0.75	0.96	ND	0.52	0.71	ND
Mean	0.95	1.47	1.44	0.67	0.92	0.81	0.47	0.66	0.79
Range	0.57, 1.27	0.86, 1.92	0.81, 2.42	0.38, 1.02	0.56, 1.26	0.54, 1.41	0.28, 0.94	0.42, 1.07	0.33, 1.73

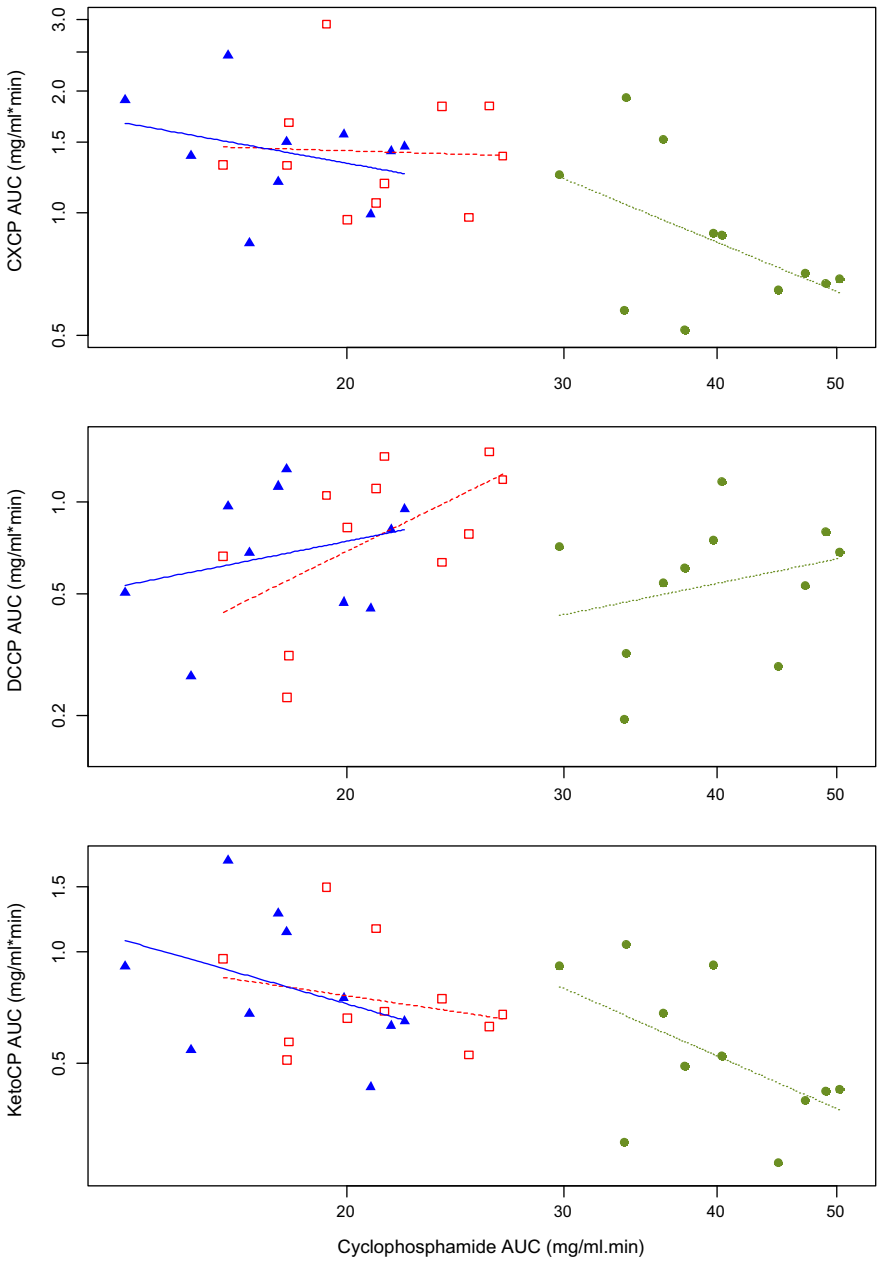

Fig. 4 – Relationship between CXCP, DCCP and KetoCP AUC and cyclophosphamide AUC on course 1 of treatment. Day 1 levels are indicated with green circles, day 2 with red squares and day 3 with blue triangles. The regression lines are shown in dotted, dashed and solid for days 1, 2, and 3, respectively.

amongst individuals.[23] In two studies it has been demonstrated that the metabolism of cyclophosphamide plays an important role in terms of both toxicity and efficacy.[8,9]

High dose sequential monotherapy followed by autologous stem cell rescue was an experimental strategy in poor risk metastatic STS. This arm of the study had to be prematurely stopped in view of the continued poor outcome.[18] This study provided an opportunity to investigate pharmacokinetics in children treated with high dose cyclophosphamide. The two reactive intermediates of cyclophosphamide (4-hydroxy cyclophosphamide and aldophosphamide) and the active metabolite phosphoramide mustard are unstable. Although bedside stabilisation techniques have been described,[30] this is not practical in multiple centres. Hence, this study measured the parent drug and the stable inactive metabolites.

Although the number of patients in this study was small, a population pharmacokinetic analysis was appropriate given the rich data and the need to characterise variability between days and courses of administration. A one-compartment model, with *CL* and *V* related to body size (SA) and variation permitted between the different days of administration provided the best fit to the data. Estimates of *CL* and of the auto-induction effect were similar to those published previously.[31] All patients showed an increase in *CL* on successive

days, although the increase was greatest (on average 88%) from day 1 to day 2. In contrast to previous reports, there was a small systematic difference in *CL* between cycles; on average *CL* on course 3 was 10% higher than on course 1 suggesting that by the start of the second course of cyclophosphamide the autoinduction which occurs during course 1 had effectively been reversed. Relative to the increase between days of treatment, the level of enzyme activity would appear to return to near pre-treatment values before the second cycle of cyclophosphamide. The AUCs of inactive metabolites were higher on day 3 compared to day 1 of cyclophosphamide treatment. The increase could be due to either accumulation of metabolites or due to autoinduction of enzymes. Interestingly there was no correlation between cyclophosphamide clearance, or AUC, and the AUCs of inactive metabolites in these high dose MMT98 patients.

Interindividual variation in *CL* (CV 25–30%, depending upon day of treatment) was largely explained by body size (*SA*) and differences between day and course of treatment. Once these factors had been taken into account IIV was substantially reduced (CV 11%). This level of variation is smaller than in previous high dose studies in both paediatric and adult populations.[32,33] Differences observed between the current findings and those from comparable studies published in children may be related to long term dexamethasone treatment in previously studied paediatric patient populations.[32] In this respect, the effect of dexamethasone on induction of key enzymes involved in the metabolism of cyclophosphamide, including CYP2B6 and CYP3A4, may add to the level of pharmacokinetic variability observed.[34] Another factor that may impact on the low interpatient variability in the current study, as compared to published data, is the larger variability in the range of cyclophosphamide doses administered in previous studies.

This study provides data on the pharmacokinetics and metabolism of cyclophosphamide in a high-dose setting. Although not successful as a therapy for rhabdomyosarcoma, similar regimens are used in other diseases and the pharmacological insight gained has clear clinical relevance. These data indicate that administration of high-dose cyclophosphamide over several days results in an increase in metabolism, possibly by induction of the activation pathway. However, following a period of approximately 4 weeks between cyclophosphamide doses, this autoinduction effect had effectively been reversed; with day 1 *CL* values on the second course of cyclophosphamide (treatment course 3) within 10% of those observed on the first cyclophosphamide course. The data also suggest that the degree of intersubject variation in cyclophosphamide elimination is not large, and is accounted for by surface area as the most relevant indicator of body size. From a clinical perspective it is also encouraging that data generated in this study would suggest minimal differences in cyclophosphamide pharmacokinetics when administered as high dose chemotherapy as compared to more conventional dosage regimens.

Conflict of interest statement

None declared.

Acknowledgement

MC and JE are supported by Cancer Research, UK.

REFERENCES

1. Patte C, Auperin A, Michon J, et al. The Societe Francaise d'Oncologie Pediatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. *Blood* 2001;**97**(11):3370–9.
2. Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. *J Clin Oncol* 1995;**13**(3):610–30.
3. Bernstein M, Kovar H, Paulussen M, et al. Ewing's sarcoma family of tumors: current management. *Oncologist* 2006;**11**(5):503–19.
4. Kushner BH, LaQuaglia MP, Bonilla MA, et al. Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. *J Clin Oncol* 1994;**12**(12):2607–13.
5. Grundy RG, Wilne SA, Weston CL, et al. Primary postoperative chemotherapy without radiotherapy for intracranial ependymoma in children: the UKCCSG/SIOP prospective study. *Lancet Oncol* 2007;**8**(8):696–705.
6. Marks DI, Forman SJ, Blume KG, et al. A comparison of cyclophosphamide and total body irradiation with etoposide and total body irradiation as conditioning regimens for patients undergoing sibling allografting for acute lymphoblastic leukemia in first or second complete remission. *Biol Blood Marrow Transplant* 2006;**12**(4):438–53.
7. Busse D, Busch FW, Bohnenstengel F, et al. Dose escalation of cyclophosphamide in patients with breast cancer: consequences for pharmacokinetics and metabolism. *J Clin Oncol* 1997;**15**(5):1885–96.
8. Yule SM, Price L, McMahon AD, Pearson ADJ, Boddy AV. Cyclophosphamide metabolism in children with non-Hodgkin's lymphoma. *Clin Cancer Res* 2004;**10**(2):455–60.
9. Ayash LJ, Wright JE, Tretyakov O, et al. Cyclophosphamide pharmacokinetics: correlation with cardiac toxicity and tumor response. *J Clin Oncol* 1992;**10**:995–1000.
10. Atra A, Pinkerton R. High-dose chemotherapy in soft tissue sarcoma in children. *Crit Rev Oncol Hematol* 2002;**41**(2):191–6.
11. Carli M, Colombatti R, Oberlin O, et al. European intergroup studies (MMT4–89 and MMT4–91) on childhood metastatic rhabdomyosarcoma: final results and analysis of prognostic factors. *J Clin Oncol* 2004;**22**(23):4787–94.
12. Maurer HM, Beltangady M, Gehan EA, et al. The Intergroup Rhabdomyosarcoma Study-I. A final report. *Cancer* 1988;**61**(2):209–20.
13. Maurer HM, Gehan EA, Beltangady M, et al. The Intergroup Rhabdomyosarcoma Study-II. *Cancer* 1993;**71**(5):1904–22.
14. Pinkerton CR. Megatherapy for soft tissue sarcomas. EBMT experience. *Bone Marrow Transplant* 1991;**7**(Suppl 3):120–2.
15. Emminger W, Emminger-Schmidmeier W, Hawliczek R, et al. High-dose melphalan, etoposide ± carboplatin (MEC) combined with 12-gray fractionated total-body irradiation in children with generalized solid tumors. *Pediatric Hematol Oncol* 1991;**8**(1):13–22.
16. Horowitz ME, Kinsella TJ, Wexler LH, et al. Total-body irradiation and autologous bone marrow transplant in the treatment of high-risk Ewing's sarcoma and rhabdomyosarcoma. *J Clin Oncol* 1993;**11**(10):1911–8.
17. Foot ABM, Pinkerton CR, Stevens M, Morland BJ, McDowell HP. Sequential rapid high dose single agent consolidation therapy for metastatic sarcoma in children. In: International Society of Pediatric Oncology SIOP XXIX Meeting, Medical and Pediatric Oncology, 1997; Istanbul, Turkey; 1997. p. 409 (abs 89).

18. McDowell HP, Foot AB, Ellershaw C, et al. Outcomes in paediatric metastatic rhabdomyosarcoma: results of The International Society of Paediatric Oncology (SIOP) study MMT-98. *Eur J Cancer* 2010;**46**(9):1588–95.
19. Chisholm JC, Machin D, McDowell H, et al. Efficacy of carboplatin given in a phase II window study to children and adolescents with newly diagnosed metastatic soft tissue sarcoma. *Eur J Cancer* 2007;**43**(17):2537–44.
20. Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. *J Pharmacokinet Pharmacodyn* 2001;**28**(3):231–52.
21. Veal GJ, Errington J, Tilby MJ, et al. Adaptive dosing and platinum-DNA adduct formation in children receiving high-dose carboplatin for the treatment of solid tumours. *Br J Cancer* 2007;**96**(5):725–31.
22. Zhang J, Tian Q, Chan SY, et al. Metabolism and transport of oxazaphosphorines and the clinical implications. *Drug Metab Rev* 2005;**37**(4):611–703.
23. de Jonge ME, Huitema ADR, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet* 2005;**44**(11):1135–64.
24. Gregory SA, Trumper L. Chemotherapy dose intensity in non-Hodgkin's lymphoma: is dose intensity an emerging paradigm for better outcomes? *Ann Oncol* 2005;**16**(9):1413–24.
25. van der Wall E, Beijnen JH, Rodenhuis S. High-dose chemotherapy regimens for solid tumors. *Cancer Treat Rev* 1995;**21**(2):105–32.
26. Szumilas P, Barcew K, Baskiewicz-Masiuk M, et al. Effect of stem cell mobilization with cyclophosphamide plus granulocyte colony-stimulating factor on morphology of haematopoietic organs in mice. *Cell Prolif* 2005;**38**(1):47–61.
27. Busse D, Busch FW, Schweizer E, et al. Fractionated administration of high-dose cyclophosphamide: influence on dose-dependent changes in pharmacokinetics and metabolism. *Cancer Chemother Pharmacol* 1998;**43**(3):263–8.
28. Bohnenstengel F, Hofmann U, Eichelbaum M, Kroemer HK. Characterization of the cytochrome P450 involved in side-chain oxidation of cylophosphamide in humans. *Eur J Clin Pharmacol* 1996;**51**(3–4):297–301.
29. Ren S, Yang JS, Kalhorn TF, Slattery JT. Oxidation of cyclophosphamide to 4-hydroxycyclophosphamide and deschloroethylcyclophosphamide in human liver microsomes. *Cancer Res* 1997;**57**(19):4229–35.
30. Kalhorn TF, Howald WN, Cole S, et al. Rapid quantitation of cyclophosphamide metabolites in plasma by liquid chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006;**835**(1–2):105–13.
31. Huitema ADR, Mathot RAA, Tibben MM, Rodenhuis S, Beijnen JH. A mechanism-based pharmacokinetic model for the cytochrome P450 drug-drug interaction between cyclophosphamide and thioTEPA and the autoinduction of cyclophosphamide. *J Pharmacokinet Pharmacodyn* 2001;**28**(3):211–30.
32. Yule SM, Foreman NK, Mitchell C, et al. High-dose cyclophosphamide for poor-prognosis and recurrent pediatric brain tumors: a dose-escalation study. *J Clin Oncol* 1997;**15**(10):3258–65.
33. Ren S, Kalhorn TF, McDonald GB, et al. Pharmacokinetics of cyclophosphamide and its metabolites in bone marrow transplantation patients. *Clin Pharmacol Ther* 1998;**64**(3):289–301.
34. Lindley C, Hamilton G, McCune JS, et al. The effect of cyclophosphamide with and without dexamethasone on cytochrome P450 3A4 and 2B6 in human hepatocytes. *Drug Metab Dispos* 2002;**30**(7):814–22.