Etoposide pharmacokinetics in children treated for acute myeloid leukemia

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We studied the pharmacokinetics of etoposide in 45 children treated for newly diagnosed acute myeloid leukemia. Etoposide, 100 mg/m^2 body surface area/24 h, was administered by 96-h continuous intravenous infusion. Concomitantly, the children received cytarabine 200 mg/m^2/24 h by intravenous infusion and 6-thioguanine 100 mg/m^2 twice daily orally. Median total body clearance in children 0.5–1.8 ($n=4$) and 2.3–17.7 years old ($n=36$) without Down's syndrome was 17.1 and 17.6 ml/min/m^2, respectively ($P=0.96$). Five children with Down's syndrome had a median clearance of 13.6 ml/min/m^2 ($P=0.067$ compared with non-Down's syndrome children). Eighteen of the children received a second identical treatment course 3–4 weeks later; there was a significant correlation between individual clearance values ($\rho=0.56$; $P=0.017$). We found no significant correlation between etoposide pharmacokinetics and the remission rate or the relapse rate. In conclusion, our findings indicate that special dose-calculation guidelines for infants above 3 months old are not substantiated by age-dependent pharmacokinetics of etoposide. Down's syndrome children might be candidates for dose reduction if our data are confirmed in larger numbers of patients. Low course-to-course variability indicates that pharmacokinetically guided dosing of etoposide might be clinically relevant, if larger studies can demonstrate that this approach decreases toxicity or increases response rates. ***Anti-Cancer Drugs*** **17:1087–1094 © 2006 Lippincott Williams & Wilkins.**

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Introduction

The epipodophyllotoxin etoposide (VP-16), which interferes with topoisomerase II activity, is widely used in pediatric oncology for the treatment of acute leukemia, Hodgkin's and non-Hodgkin's lymphoma, sarcoma, germ cell tumor, neuroblastoma, and brain tumors. The dose-limiting toxicity is myelosuppression. Compared with many other anticancer drugs, the pharmacokinetics of etoposide have been well studied in both adults and children (for reviews, see Henwood and Brogden [1] Groninger *et al.* [2]). A number of points, however, still remain to be addressed. Only limited data are available for infants, an age group in which the dosing of drugs often is a problem [3]. Children with Down's syndrome (DS), who constitute 10–15% of children diagnosed with acute myeloid leukemia (AML), have a higher morbidity than non-DS children after treatment with some anticancer drugs [4,5]. This might partly be due to differences in drug distribution or elimination, but for etoposide such data have only been published for two DS patients [6].

In the treatment of AML, etoposide is generally given concomitantly with other antineoplastic agents. The effect of such combinations on etoposide pharmacokinetics is largely unknown, although some studies indicate that potentially nephrotoxic drugs can affect etoposide pharmacokinetics [7–9]. The most crucial lack of knowledge, however, concerns the correlation between dose, plasma concentrations and clinical outcome. In adult patients with small cell lung cancer, a correlation between systemic drug exposure and response has been demonstrated for etoposide, but in other studies no such relationship was shown [10]. For childhood malignancies, we found no such investigations concerning etoposide [1,2]. In a study that compared conventional to individualized chemotherapy for childhood acute lymphoblastic leukemia, no correlation between pharmacokinetics and effect were found for teniposide, another epipodophyllotoxin [11].

The aim of the present investigation was to study the pharmacokinetics of etoposide in children with AML,

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treated according to a common protocol at Nordic centers for pediatric oncology. As etoposide is highly protein bound and the degree of hematological toxicity has been reported to correlate better to unbound drug than to total drug [12,13], we measured the concentration of both total and free etoposide. In a subgroup of patients, we repeated the pharmacokinetic studies during an identical second treatment course to examine the intraindividual variability. Pharmacokinetic data were correlated to clinical effect, estimated both by bone marrow morphology after remission induction therapy and by long-term clinical follow-up.

Patients

Between March 1995 and October 2000, 45 children were successfully included in the study at eight Nordic centres for pediatric oncology: Copenhagen, Helsinki, Linköping, Lund, Tampere, Oslo (Ullevål), Umeå and Uppsala. During this time period, 87 children were diagnosed with AML, i.e. our patient material represents 52% of the patient population at these centers. Reasons for not including patients were mostly practical difficulties, such as lack of extra venous access or lack of staff to handle research samples, or sometimes refusal of patients or parents to participate. One batch of samples from 13 patients was destroyed during transportation.

Five of the children had DS. DS children were younger than the non-DS children, median age 1.9 and 10.3 years, respectively. As expected, the DS children also differed in their distribution to FAB type (Table 1). As AML in children with DS differs markedly from other forms of AML, the two groups are analyzed separately. All children were treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) AML-93 protocol [14] and studied during the first induction course. As shown in Fig. 1, this course included an intrathecal injection of methotrexate on day 1, followed by etoposide, 100 mg/m^2 body surface area (BSA)/24 h, and cytarabine, 200 mg/m^2/24 h, administered concomitantly by constant infusion pump over a 96-h period on days 1–4. They were dissolved in 0.9% NaCl or glucose 50 g/l to give an etoposide concentration of 0.4 mg/ml. During the same 96-h period, 100 mg/m^2 of 6-thioguanine was administered orally every 12 h to a total dose of 800 mg/m^2. On day 5, doxorubicin 75 mg/m^2 was given as an 8-h infusion. Data on other drugs administered, e.g. antiemetics, analgesics and antibiotics, were not available to us. According to the treatment protocol, BSA was calculated by the formula $m^2 = \sqrt{[\text{height (cm)} \times \text{weight (kg)}/3600]}$ for children ≥ 2 years of age and $m^2 = \text{weight (kg)}/30$ for children < 2 years old. Thus, infants received 3.3 mg etoposide per kg body weight.

Table 1 Patient characteristics

	Non-DS	DS	*P*-value
No.	40	5	
Age (years)			
median	10.3	1.9	0.004
range	0.5–17.7	1.2–3.4	
Sex (*n*)			
male	17	1	
female	23	4	
WBC (10^9/l)			
median	15.5	12.3	0.66
range	0.5–126	7.1–44.7	
FAB (*n*)			
M0	3	0	
M1	7	2	
M2	10	0	
M3	1	0	
M4	9	0	
M5	7	0	
M7	1	3	
Other	2	0	

DS, Down's syndrome; WBC, white blood cell count.

According to the protocol, a bone marrow sample was drawn 3 weeks after the start of the induction course (median 24 days, range 13–42 days) to evaluate treatment response. Less than 5% blast cells in a stained smear of a nonhypoplastic bone marrow was the main criterion for complete remission (CR). If the first bone marrow was too hypoplastic to determine remission, bone marrow samples were to be obtained at weekly intervals until normal hemopoesis or regrowth of malignant cells emerged. Twenty-nine out of the 45 patients reached CR after the initial course and received a second treatment course identical to the one given up-front. Repeated sampling for pharmacokinetic analysis was successful in 18 of the 29 patients who received two identical treatment courses (one of them with DS). Patients not in CR after the first course received treatment with cytarabine and mitoxantrone. After two induction courses, all patients who had reached CR received a total of four consolidations. The backbone of this treatment was high-dose cytarabine, administered as single drug (one course), or combined with etoposide (two courses) or mitoxantrone (one course) [14]. Children with a matched related donor were candidates for allogeneic stem cell transplantation (SCT).

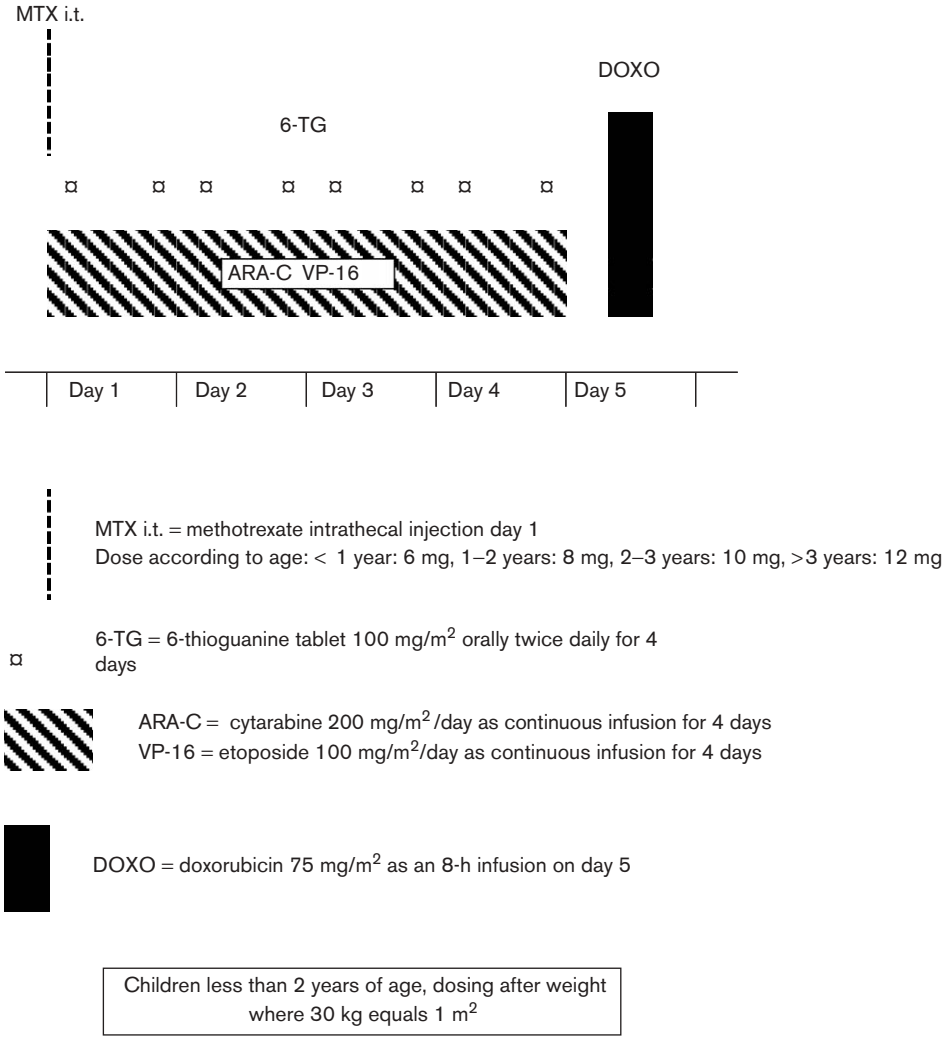
Patient characteristics and clinical follow-up data were obtained from annual reports submitted from the treating clinicians to the Nordic registry at the Childhood Cancer Research Unit in Stockholm and the last day of follow-up was 31 December 2004. Toxicity has not been routinely reported to the registry.

Local ethics committees approved the study.

Plasma samples

Blood samples were drawn before, and 48, 72 and 95 h after start of the etoposide infusion, i.e. the last sample was drawn 1 h before the infusion was completed. Blood

Fig. 1



Induction course of the Nordic Society of Paediatric Haematology and Oncology (NOPHO) acute myeloid leukemia-93 (AML-93) protocol.

was drawn from a venous line not used for etoposide infusion and collected in tubes containing ethylene diaminetetraacetic acid.

For a number of patients ($n = 29$), the doxorubicin plasma level was measured during the doxorubicin infusion on day 5, and steady-state concentration and total body clearance were calculated as reported in a previous study [15].

Patient data (body weight, height, actual dose administered), as well as exact times for start and stop of infusions, and for blood sampling, were noted. Serum concentrations of creatinine, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), determined before the start of the induction course, were also recorded.

Analytical procedure

Etoposide concentrations were determined by high-performance liquid chromatography (HPLC). Plasma samples were thawed and 0.5 ml was used for analysis. After the addition of teniposide (5 μg) as internal standard and liquid extraction with chloroform, the organic phase was evaporated under nitrogen. The residue was redissolved in 1 ml water/methanol (50/50). The extract was injected (25 μl) into the HPLC system. A reversed-phase system with a Nucleosil column 7 μm ($150 \times 4.6\,mm^2$) equipped with a NewGuard Phenyl precolumn eluted with methanol/water/acetonitrile/acetic acid (43/52/4/1) at a flow rate of 1.0 ml/min was used to separate etoposide from endogenous compounds. Quantitation was performed using electrochemical detection. The signal was integrated using peak area ratios [16].

Free concentrations of etoposide were determined after removal of plasma proteins by ultrafiltration on Millipore Centrifree filters. Subsequently, 50 µl of the ultrafiltrate was injected directly into the HPLC system [13].

Pharmacokinetic evaluation and statistics

On the basis of recorded data for body weight and height, we recalculated the BSA of all patients by the formula $m^2 = \sqrt{[\text{height (cm)} \times \text{weight (kg)}/3600]}$. Body mass index was calculated as weight/(height)^2. Plasma clearance (Cl) was calculated according to the formula $Cl = D/T/C_{ss}$, where D/T is the actual dose rate and C_{ss} is the observed steady-state concentration of the drug.

The Spearman rank test (two-sided) was used to examine correlations, the Mann–Whitney *U*-test to compare values from two groups, the Kruskall–Wallis test to examine differences between three or more groups, the Wilcoxon signed rank test to compare two related samples, the Friedman test to examine several related samples and logistic regression analysis to test the probability of a defined event. For linear regression analysis, a natural log transformation of one (univariate) or several covariates (multivariate analyses) was performed. The SPSS 12.0 software package (SPSS, Chicago, Illinois, USA) was used for the calculations. $P < 0.05$ was considered as statistically significant.

Results

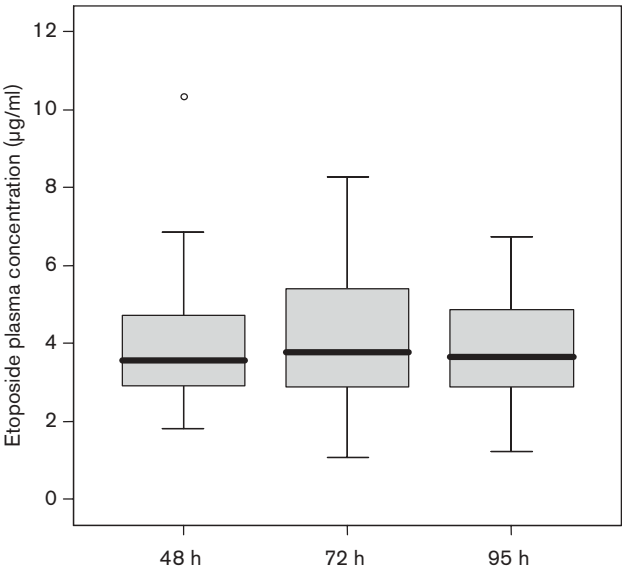
No statistically significant difference exists, or any trend to a difference, between etoposide concentrations measured 48, 72 and 95 h after the start of the infusion ($P = 0.54$, see Fig. 2). The same was true for the concentrations of free etoposide and the percentage of free etoposide (not shown). For each individual, we used the mean value of these three observations as the steady-state concentration of the drug in the subsequent calculations.

Children without Down's syndrome

The median etoposide dose received by children ≥ 2 years of age (range 2.3–17.7 years) was 99.9 mg/m^2/24 h days 1–4, which was very close to the target dose of 100 mg/m^2/24 h. The median dose received by children < 2 years of age (range 0.5–1.8 years) was 75.8 mg/m^2/24 h ($P = 0.003$), corresponding to a dose of 3.41 mg/kg/24 h (range 3.33–3.57).

The median steady-state concentration of etoposide was 4.00 µg/ml in children aged ≥ 2 years and 3.03 µg/ml in children < 2 years old ($P = 0.055$; Table 2). The median concentration of free etoposide was 0.12 and 0.14 µg/ml in these groups, respectively. Median values for free etoposide calculated as percentage of total etoposide were 3.1 and 4.2%. Median total body clearance was very similar in the two age groups, 17.6 and 17.1 ml/min/m^2 in children above and below 2 years of age, respectively. The four youngest children aged 0.5, 0.6, 1.0 and 1.8 years had clearance values of 17.2, 16.4, 17.1 and 19.8 ml/min/m^2, respectively.

Fig. 2



Etoposide plasma concentration (µg/ml) measured 48, 72 and 95 h after the start of a 96-h constant infusion. The box-and-whisker plot shows median, first and third quartiles; whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles.

Total body clearance was used to explore the correlation between pharmacokinetics and background variables, and all non-DS children were included in this analysis. No difference exists between boys and girls. In a monovariate analysis, clearance was significantly correlated to ALT ($\rho = -0.33$; $P = 0.038$), while age, weight, height, body mass index, AST, creatinine, albumin, white blood cell (WBC) count at diagnosis and dosage in mg/m^2 were nonsignificant. When tested in linear regression analysis after log transformation of the clearance and ALT values, no significant correlation was found ($P = 0.26$) and the predictive value of ALT levels was low ($R^2 = 0.03$).

Most children had normal or near-normal ALT, AST, albumin, and creatinine values at the start of therapy. Three children with ALT values above 2 times the upper normal limit had clearance values of 11.3, 13.9 and 16.1 ml/min/m^2. Only one child had a plasma creatinine above 1.5 times the upper normal limit, and this child had an etoposide clearance of 6.4 ml/min/m^2, the second lowest value recorded.

Doxorubicin clearance values were available for 29 of the patients. No significant correlation exists between the

Table 2 Summary of pharmocokinetic parameters in children with or without Down's syndrome (DS)

	Non-DS <2 years	P^a	Non-DS >2 years	P^b	DS
No.	4		36		5
Dose (mg/m^2/24 h)					
median	75.8	(0.003)	99.9	(0.001)	66.2
range	68.1–86.5		49.4–107.5		45.3–84.4
P 25–75			97.5–101.7		
Steady-state conc (μg/ml)					
median	3.03	(0.055)	4.00	(0.27)	3.37
range	2.75–3.25		1.65–10.6		2.51–4.25
P 25–75			3.16–5.03		
Clearance (ml/min/m^2)					
median	17.1	(0.96)	17.6	(0.067)	13.6
range	16.4–19.8		5.2–41.7		7.6–19.7
P 25–75			13.7–22.1		
Clearance (mlmin/kg)					
median	0.79	(0.12)	0.62	(0.81)	0.65
range	0.72–0.87		0.14–1.30		0.36–0.84
P 25–75			0.45–0.80		
Free etoposide (μg/ml)					
median	0.14		0.12		0.07
range	0.06–0.21		0.0–0.31		0.05–0.07
P 25–75			0.08–0.14		
Free etoposide (%)					
median	4.2		3.1		1.49
range	2.0–6.5		0.0–6.8		1.24–2.53
P 25–75			1.7–4.1		

[a]Non-DS <2 years vs. >2 years.
[b]DS vs. all non-DS children.
P 25–75 = 25th–75th percentiles.
Free etoposide and etoposide % were only measured in 2 non-DS children <2 years 27 non-DS children >2 years and three DS children.

total body clearance of etoposide and doxorubicin ($\rho = 0.27$; $P = 0.16$).

Children with Down's syndrome

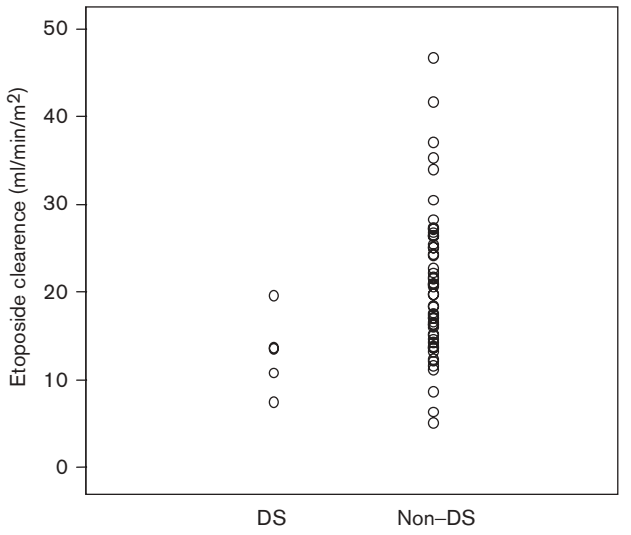
Five children with DS were studied: 1.2, 1.8, 1.9, 2.3 and 3.4 years old, respectively. They received a median etoposide dose of 66.2 mg/m^2/24 h (Table 2). The median etoposide clearance of the DS children was 13.6 ml/min/m^2, a value about 20% lower than in non-DS children ($P = 0.067$; Fig. 3). Free etoposide was measured in three out of the five children and the values were of the same magnitude as in non-DS children.

Repeated courses

Repeated sampling was successful in 18 patients (one with DS) receiving a second treatment course identical to the first one. The median interval between the start of the courses was 23 days (range 18–42 days). Median clearance was 16.2 (25th–75th percentiles 13.7–21.4) and 15.9 (14.2–21.7) ml/min/m^2 for courses 1 and 2, respectively. As displayed in Fig. 4, most patients showed little variability from course-to-course and the correlation between the clearance values from the two courses was high ($\rho = 0.56$; $P = 0.017$). The percentage of free etoposide was also similar, with median values of 2.8 and 3.3%, respectively ($\rho = 0.82$; $P = 0.001$; $n = 13$).

No significant correlation was found between etoposide clearance measured during course 2 and any of the background variables mentioned above (body composition and biochemical variables were re-tested before the start of course 2, data not shown).

Fig. 3

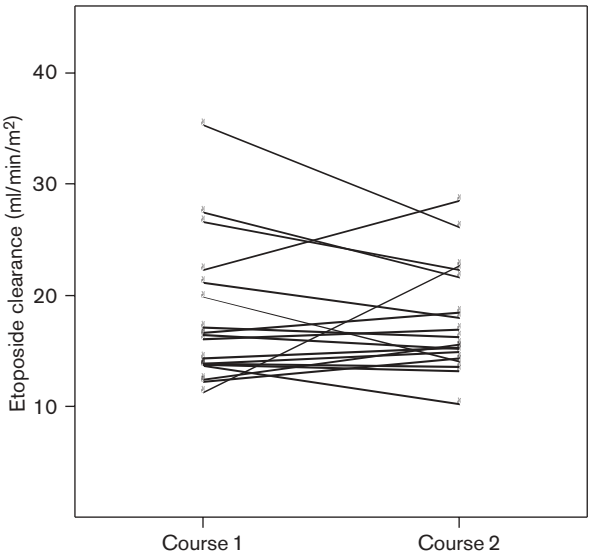


Etoposide total body clearance in children with ($n = 5$) and without ($n = 40$) Down's syndrome.

Pharmacodynamics in non-Down's syndrome children

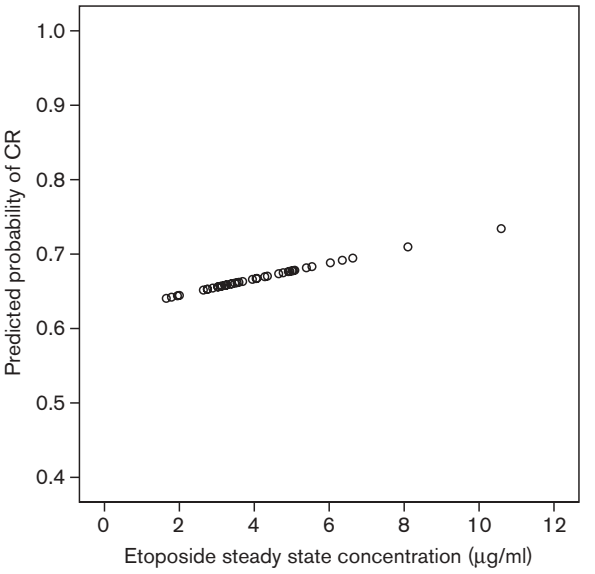
One patient died from aplasia 1 month after the start of treatment, before any evaluation of treatment response, and is therefore not included in the calculations below. He had an etoposide clearance of 8.7 ml/min/m^2 and a

Fig. 4



Etoposide total body clearance in patients treated by two identical courses with a 3- to 4-week interval ($n=18$). Lines connect individual values.

Fig. 5



Predicted probability of complete remission (CR) after induction therapy as a function of etoposide concentration ($n=39$). Children with Down's syndrome are excluded. The plot illustrates the lack of a significant concentration–effect relationship within the concentration interval studied.

steady-state concentration of 8.1 µg/ml, the second highest value recorded in any patient. This might have contributed to the severe aplasia, but to what extent other factors contributed could not be determined.

We compared 26 patients who went into CR after the first treatment course with the 13 patients who did not. They showed no significant difference, or any trend to a difference, in etoposide steady-state concentrations or clearance values ($P = 0.94$ and 0.85, respectively). The same was true for the concentrations of free etoposide and the percentage of free etoposide (tested in 18 CR and 10 non-CR patients). Etoposide steady-state concentration was not an independent factor for CR in univariate ($P = 0.82$) or multivariate regression analysis including sex, age and WBC count ($P = 0.71$). Figure 5 shows the predicted probability of CR as a function of etoposide concentration in a univariate analysis. In a multivariate analysis including also doxorubicin steady-state concentrations ($n = 29$), doxorubicin concentration tended to be an independent factor ($P = 0.07$), with a weaker trend for age ($P = 0.12$).

Twenty patients were in continuous CR at the latest follow-up (seven after allogeneic SCT in first CR), while 17 had relapsed (four after allogeneic SCT in first CR), with a median follow-up time of 7.6 years (range 4.5–9.8 years). Two patients died in CR after allogeneic SCT. No statistically significant differences were observed between CR and relapsed patients for etoposide plasma concentration or total body clearance measured during the first induction course ($P = 0.68$ and 0.94, respectively).

Treatment of a very young non-study patient

We recently treated an infant with AML at 7 weeks of age. She received an etoposide dose of 3 mg/kg/24 h, corresponding to 54 mg/m^2/24 h, administered together with cytarabine 6 mg/kg/24 h. After 70-h constant infusion, the plasma concentration of etoposide was 3.43 µg/ml. The total body clearance calculated from this single sample was 11.0 ml/min/m^2. She had normal aminotransferases and bilirubin values, but her glomerular filtration rate was 47 ml/min/1.73 m^2, on the basis of determinations of plasma cystatin C. The patient is alive and well 1.5 years later.

Discussion

We measured the etoposide plasma concentration at 48, 72 and 95 h during a 96-h constant infusion. Steady state was reached before 48 h, as evidenced by very stable plasma levels throughout the sampling period. This was expected, as the terminal half-life of etoposide in plasma is short, ranging between 2 and 6 h in children [8,17–19]. Etoposide is known to be highly bound to plasma proteins and our finding that only 3–4% of total etoposide was in free form agrees with previous reports [20,21].

Most children ≥ 2 years old received etoposide in amounts very close to the target dose, 100 mg/m^2/24 h, while the median dose of four children < 2 years of age

was only 75.8 mg/m^2/24 h. This was a consequence of the dosing rules of the NOPHO AML-93 protocol, which prescribed a dose based on BSA in children ≥ 2 years of age, but an etoposide dose of 3.3 mg/kg body weight in infants < 2 years. The median steady-state concentration in the infants was 76% of that found in children ≥ 2 years old, indicating that they received a less intense treatment. Median total body clearance of etoposide was very similar in the two age groups and all four infants had clearance values close to the median clearance of older children, also the two youngest aged 0.5 and 0.6 years, respectively. This agrees with the data of Boos *et al.* [19] and Eksborg *et al.* [6], who found no difference in etoposide pharmacokinetics between children and infants, even in the age range of 3–12 months.

Many contemporary protocols recommend dose reduction of etoposide for children < 1 year of age, sometimes with additional reduction for infants < 6 months old, Interfant 99 and Interfant 05, International collaborative treatment protocols for infants under 1 year with acute lymphoblastic leukemia, study coordinator, R. Pieters, Sophia Children's Hospital, Rotterdam, The Netherlands, and NOPHO-AML 2004 study, chairman H. Hasle, Skejby Hospital, Aarhus, Denmark [14]. We think available data support the idea that infants > 3 months old should receive etoposide in doses calculated from BSA as in children > 1 year of age. Dose reduction results in low plasma levels and this might be one of the reasons why infants treated for ALL have an inferior prognosis, especially those < 6 months of age.

Children < 3 months old represent a special problem, as renal function is immature at birth, with a gradual maturation during the first weeks and months [22]. We administered approximately two-thirds of the calculated dose based on BSA to a 7-week-old girl not treated within the time frame of the study. This resulted in an 'ordinary' plasma level of etoposide and no unexpected toxicity.

Previous publications have described that etoposide elimination was decreased by cyclosporin and nephrotoxic drugs such as cisplatin and carboplatin (see reviews) [1,2]. Prednisone, on the other hand, strongly induced etoposide clearance, probably by its effect on CYP3A4-mediated metabolism of etoposide [23]. The clearance values found in our patient material were similar to those reported in a number of previous studies [8,17–19], indicating that etoposide pharmacokinetics were not significantly influenced by the concomitant administration of cytarabine and 6-thioguanine.

Renal excretion accounts for about 45% of systemic etoposide clearance and renal impairment affects etoposide pharmacokinetics [8]. Hepatic metabolism also plays an important role [18]. We found no correlation between etoposide clearance and creatinine or aminotransferase levels, but this was probably due to the fact that few patients had values outside the reference intervals. Still, the small number of children with clearly elevated creatinine or aminotransferase levels tended to have lower than average etoposide clearance values.

Children with DS have an increased risk of developing acute leukemia, especially AML, in which they constitute 10–15% of all children with this diagnosis. Several groups, including NOPHO, have reported that DS children with AML have an excellent prognosis if actively treated [14,24–28]. Still, however, much uncertainty exists about the optimal dosing of drugs administered in multiagent treatment courses, as the effect and toxicity of individual drugs are very difficult to evaluate. To our knowledge, data on etoposide pharmacokinetics have only been published for two children with DS [6]. The terminal half-life of etoposide plasma concentrations of these two children was similar to that of 14 non-DS children, but data must be interpreted with caution because the children in that study received widely ranging doses of etoposide in varying combinations with other antineoplastic agents. The NOPHO AML-93 protocol had no recommendations for dosage of etoposide in DS children, but we found that the DS patients studied here in practice received considerably reduced doses. The five DS children, aged 1.2–3.4 years, had a median etoposide clearance that was about 20% lower than in non-DS children and this resulted in plasma concentrations similar to those found in non-DS children receiving full doses.

A relationship between etoposide pharmacokinetics and response has apparently not been reported in children till now [2,29]. We compared patients who went into CR after one induction course with those who did not. No difference was found in etoposide steady-state concentrations or total body clearance, and no difference in the concentration of free etoposide or the percentage of free etoposide. Etoposide steady-state concentration was not an independent factor for CR in univariate or multivariate regression analysis. We also compared patients who remained in continuous CR at long-time follow-up with those who relapsed and again there were no differences, or any trend to differences, in pharmacokinetic parameters.

We have recently published data showing that the doxorubicin plasma concentration was an independent factor for CR in children with AML treated according to NOPHO AML-93. Twenty-nine of those children are included in the present study, and thus have pharmacokinetic data for both doxorubicin and etoposide. Although we could not show here any relationship between etoposide pharmacokinetics and response, it is important to realize that such a relationship still may exist. For most patients, the steady-state plasma concentration of

etoposide was in a rather narrow range, 3–6 μg/ml, which might represent a flat part of the concentration–effect curve. The therapeutic level of etoposide in childhood AML is not known. It can be speculated that etoposide, which is a topoisomerase II inhibitor like doxorubicin, reached a critical threshold level in most patients, with a permissive effect enabling doxorubicin to exert a dose-dependent cell kill when administered after the etoposide infusion. When etoposide is administered as single drug, or when it is included in other drug combinations, different dose–effect relationships might exist and the same is true for treatment of other malignancies than AML.

In summary, our findings, together with literature data, indicate that special dose-calculation guidelines for infants >3 months old are not substantiated by age-dependent pharmacokinetics of etoposide. A single observation suggests that dose reduction might be needed in children <3 months old. Children with DS tended to have lower clearance than non-DS children and might be candidates for dose reduction, but our data need to be confirmed in larger number of patients. We found a limited course-to-course variability, indicating that pharmacokinetically guided dosing of etoposide might be clinically relevant, if it can be demonstrated that this approach increases response or decreases toxicity without jeopardizing the antitumoral effect. We were, however, unable to demonstrate any correlation between etoposide pharmacokinetics and clinical response.

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References

1 Henwood JM, Brogden RN. Etoposide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in combination chemotherapy of cancer. *Drugs* 1990; **39**:438–490.
2 Groninger E, Proost JH, de Graaf SS. Pharmacokinetic studies in children with cancer. *Crit Rev Oncol Hematol* 2004; **52**:173–197.
3 Woods WG, O'Leary M, Nesbit ME. Life-threatening neuropathy and hepatotoxicity in infants during induction therapy for acute lymphoblastic leukemia. *J Pediatr* 1981; **98**:642–645.
4 Blatt J, Albo V, Prin W, Orlando S, Wollman M. Excessive chemotherapy-related myelotoxicity in children with Down syndrome and acute lymphoblastic leukaemia. *Lancet* 1986; **2**:914.
5 Garre ML, Relling MV, Kalwinsky D, Dodge R, Crom WR, Abromowitch M, *et al.* Pharmacokinetics and toxicity of methotrexate in children with Down syndrome and acute lymphocytic leukemia. *J Pediatr* 1987; **111**:606–612.
6 Eksborg S, Soderhall S, Frostvik-Stolt M, Lindberg A, Liliemark E. Plasma pharmacokinetics of etoposide (VP-16) after i.v. administration to children. *Anticancer Drugs* 2000; **11**:237–241.
7 Relling MV, McLeod HL, Bowman LC, Santana VM. Etoposide pharmacokinetics and pharmacodynamics after acute and chronic exposure to cisplatin. *Clin Pharmacol Ther* 1994; **56**:503–511.
8 Lowis SP, Pearson AD, Newell DR, Cole M. Etoposide pharmacokinetics in children: the development and prospective validation of a dosing equation. *Cancer Res* 1993; **53**:4881–4889.
9 Rodman JH, Murry DJ, Madden T, Santana VM. Altered etoposide pharmacokinetics and time to engraftment in pediatric patients undergoing autologous bone marrow transplantation. *J Clin Oncol* 1994; **12**:2390–2397.
10 Gruber A, Liliemark E, Tidefelt U, Paul C, Bjorkholm M, Peterson C, *et al.* Pharmacokinetics of mitoxantrone, etoposide and cytosine arabinoside in leukemic cells during treatment of acute myelogenous leukemia: relationship to treatment outcome and bone marrow toxicity. *Leuk Res* 1995; **19**:757–761.
11 Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N Engl J Med* 1998; **338**:499–505.
12 Stewart CF, Arbuck SG, Fleming RA, Evans WE. Relation of systemic exposure to unbound etoposide and hematologic toxicity. *Clin Pharmacol Ther* 1991; **50**:385–393.
13 Liliemark E, Herngren L, Pettersson B, Peterson C, Liliemark J. Ultrafiltration and subsequent high performance liquid chromatography for *in vivo* determinations of the protein binding of etoposide. *Cancer Lett* 1996; **106**:91–96.
14 Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, *et al.* Treatment stratification based on initial *in vivo* response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials. *Br J Haematol* 2003; **122**:217–225.
15 Palle J, Frost BM, Peterson C, Gustafsson G, Hellebostad M, Kanerva J, *et al.* Doxorubicin pharmacokinetics is correlated to the effect of induction therapy in children with acute myeloid leukemia. *Anticancer Drugs.* 2006; **17**:385–392.
16 Zhou R, Frostvik-Stolt M, Liliemark E. Determination of etoposide in human plasma and leukemic cells by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl* 2001; **757**:135–141.
17 Kato Y, Nishimura S, Sakura N, Ueda K. Pharmacokinetics of etoposide with intravenous drug administration in children and adolescents. *Pediatr Int* 2003; **45**:74–79.
18 Evans WE, Sinkule JA, Crom WR, Dow L, Look AT, Rivera G. Pharmacokinetics of Teniposide (VM26) and etoposide (VP16–213) in children with cancer. *Cancer Chemother Pharmacol* 1982; **7**:147–150.
19 Boos J, Real E, Schulze W, Wolff J, Euting T, Jurgens H. Investigation of the variability of etoposide pharmacokinetics in children. *Int J Clin Pharmacol Ther Toxicol* 1992; **30**:495–497.
20 Stewart CF, Pieper JA, Arbuck SG, Evans WE. Altered protein binding of etoposide in patients with cancer. *Clin Pharmacol Ther* 1989; **45**:49–55.
21 Evans WE, Rodman JH, Relling MV, Crom WR, Rivera GK, Pratt CB, *et al.* Concept of maximum tolerated systemic exposure and its application to phase I–II studies of anticancer drugs. *Med Pediatr Oncol* 1991; **19**:153–159.
22 Alcorn J, McNamara PJ. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clin Pharmacokinet* 2002; **41**:959–998.
23 Kishi S, Yang W, Boureau B, Morand S, Das S, Chen P, *et al.* Effects of prednisone and genetic polymorphisms on etoposide disposition in children with acute lymphoblastic leukemia. *Blood* 2004; **103**:67–72.
24 Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. *Leukemia* 2005.
25 Craze JL, Harrison G, Wheatley K, Hann IM, Chessells JM. Improved outcome of acute myeloid leukaemia in Down's syndrome. *Arch Dis Child* 1999; **81**:32–37.
26 Kojima S, Sako M, Kato K, Hosoi G, Sato T, Ohara A, *et al.* An effective chemotherapeutic regimen for acute myeloid leukemia and myelodysplastic syndrome in children with Down's syndrome. *Leukemia* 2000; **14**:786–791.
27 Lange BJ, Kobrinsky N, Barnard DR, Arthur DC, Buckley JD, Howells WB, *et al.* Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: children's Cancer Group Studies 2861 and 2891. *Blood* 1998; **91**:608–615.
28 Ravindranath Y, Abella E, Krischer JP, Wiley J, Inoue S, Harris M, *et al.* Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. *Blood* 1992; **80**:2210–2214.
29 Arnaout MK, Radomski KM, Srivastava DK, Tong X, Belt JR, Raimondi SC, *et al.* Treatment of childhood acute myelogenous leukemia with an intensive regimen (AML-87) that individualizes etoposide and cytarabine dosages: short- and long-term effects. *Leukemia* 2000; **14**:1736–1742.