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Report

Population pharmacokinetics of high-dose etoposide in children receiving different conditioning regimens

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Pharmacokinetics after high-dose (HD) etoposide (Eto) (40 mg/kg i.v. once as 4-h infusion, one patient 20 mg/kg i.v. as 4-h infusion, for 3 consecutive days) were studied in 31 children and young adults (age 0.8–23.7 years, median: 8.0 years) undergoing bone marrow transplantation after different conditioning regimens. Blood samples were collected until 97 h after the end of infusion. The population analysis of the first part of data (112 samples/21 patients, well documented) served to establish the pharmacokinetic model. The same data combined with the second part of data (50 samples/10 patients, 'intention to treat') then served to calculate the final population model. Data were best described by a three-compartment model with $t_{1/2\alpha}$=0.28 h ± 3.2%, $t_{1/2\beta}$=3.6 h ± 16.9% and $t_{1/2\gamma}$=44.2 h ± 56.5%, respectively ($mean_{geom} \pm CV_{geom}$). Clearance (CL) was 15.5 ml/min/m^2 ± 30.6% ($mean_{geom} \pm CV_{geom}$) and thus at the lower range of data reported in the literature. The fraction of unbound Eto (f_u) was 7.0% (4.3–11.9%) [median (range)], with high intra-individual variability. An increase in f_u with increasing total Eto was observed. The question of a principally lower Eto CL in children, as compared to adults, after HD treatment remains open. [© 2002 Lippincott Williams & Wilkins.]

Key words: Bone marrow transplantation, children, etoposide, high dose, P-Pharm, population pharmacokinetics.

Introduction

As many conditioning regimens contain high-dose (HD) etoposide (Eto), there is considerable interest in its pharmacokinetics. In a variety of studies, linearity of pharmacokinetics for Eto from low-dose (LD) to HD schedules was observed in adults. There was no difference between the kinetics in adults and children at lower doses.[1] Data on the kinetics in children under HD conditions are, however, limited.[2–4]

Based on clinical drug monitoring data, we studied Eto pharmacokinetics in 31 children and young adults undergoing bone marrow transplantation (BMT) (stage IV neuroblastoma and a variety of other malignant diseases). The main objective of the monitoring procedure was to examine the pharmacokinetics of Eto, with a special focus on terminal elimination. Due to the limited number of samples per patient, population pharmacokinetic analysis was performed. Concentration dependency and variability of protein binding were investigated.

Materials and methods

Patients and treatment regimen

Thirty-one patients with different diagnoses underwent dose therapeutic drug monitoring and BMT at the University Children's Hospital Tübingen. Demographic data of the patients are given in Table 1. Conditioning consisted of: protocol 1: Eto (40 mg/kg), busulfan (divided doses daily for 4 days, total dose: 16–20 mg/kg), cyclophosphamide (twice 60 mg/kg) and ALG (for 3 days, total dose: 60 mg/kg); protocol 2: Eto (40 mg/kg), total body irradiation (12 Gy for 3 consecutive days) and cyclophospha-

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Table 1. Patient characteristics

Patient	Age (years)	Sex	Protocol	Dose (mg/kg)	Diagnosis
1	0.8	F	1	40.0	M Farquahr
2	4.3	F	3	40.0	NBL IV
3	10.0	M	3	40.0	NBL IV
4	3.5	F	2	36.9	CML
5	8.9	M	3	40.0	RMS IV
6	18.7	F	2	40.0	CML
7	4.1	M	3	39.9	NBL IV
8	3.5	F	3	40.0	NBL IV
9	1.5	M	3	40.0	NBL IV
10	13.3	F	2	40.0	CML
11	6.2	F	2	40.0	CML
12	8.8	M	3	40.0	NBL IV
13	2.6	M	3	40.4	NBL IV
14	8.7	M	3	40.0	NBL IV
15	8.0	M	2	40.0	ALL
16	4.1	M	3	38.5	NBL IV
17	14.0	M	2	37.9	ALL
18	3.2	M	3	20.0	NBL III
19	13.9	M	2	41.9	Pre-B-ALL, 2.rec.
20	14.0	F	2	40.0	ALL
21	8.3	F	3	38.1	NBL IV
22	9.0	M	3	40.0	NBL IV
23	2.0	M	1	39.4	AML
24	10.6	M	5	25.3[a]	B-ALL
25	23.7	M	2	39.6	CML
26	6.4	M	3	32.0	nephroblastoma
27	3.3	M	3	40.0	NBL III
28	10.8	M	1	39.0	AML
29	3.3	F	2	40.0	Ki1 NHL
30	7.7	M	3	40.0	NBL IV
31	8.9	M	3	38.1	NBL IV
Median	8.0			40.0	
Minimum	0.8			20.0	
Maximum	23.7			41.9	

Group A: patients 1–21; group B: patients 22–31.
[a]Three applications.

mide (twice 60 mg/kg); protocol 3: Eto (40 mg/kg), melphalan (for 4 days, total dose: 180 mg/m^2) and carboplatin (for 3 days, total dose: 1500 mg/m^2); protocol 5: Eto (25 mg/kg), busulfan (divided doses daily for 4 days, total: 16-20 mg/kg) and thiotepa (900 mg/m^2). Most patients of protocol 1, 2 and 5 received cyclosporin A (CSA; 3–6 mg/kg). Thirty of 31 patients received a single dose of Eto of theoretically 40 mg/kg/4 h at day −4, the only patient treated according to protocol 5 received doses of 25 mg/kg/4 h Eto on 3 consecutive days at days −8 to −6.

Patients were divided into two groups: group A included patients with well-documented precise dosage, duration of infusion, clinical and personal data (21 patients, 112 samples), while patients with incomplete documentation accompanying the samples were evaluated in group B ('intention to treat') (10 patients, 50 samples). The number of measurements available for each patient ranged from 2 to 8 (median 5) measurements, sampling time was up to 97 h after the end of infusion. Informed consent to the blood sampling was obtained from all patients and/or their parents.

Analytical method

Eto (molecular weight: 588.6) was determined by reversed phase high-performance liquid chromatography and electrochemical detection at 0.820 V according to a method described elsewhere.[5] The plasma unbound fraction was determined after ultrafiltration of plasma using a Micron YM 10 filter (Amicon, Witten, Germany). Serum was centrifuged for 10 min at 10 000 r.p.m., and an aliquot of the filtrate was diluted with an equal amount of a mixture of water, methanol, acetonitrile and glacial

acetic acid (48:42:8:2) prior to injection. The limit of quantification was 25 μg/l and the coefficient of variation for intra-assay variability was less than 7%.

Pharmacokinetic analysis

The pharmacokinetic program P-Pharm (version 1.5) was used to determine the population parameters of Eto. The distribution of the random effect was assumed to be normal, initial estimates were obtained by stripping of individual data. The evaluation criteria included: the log-likelihood value, the Akaike criterion, the relative residual plot and the examination of the change of the remaining inter-individual variability in the fixed effect parameters. The pharmacokinetic model was evaluated using plasma data from group A, while data sets A and B were combined to obtain the final pharmacokinetic parameters. Derived pharmacokinetic parameters such as half-lives ($t_{1//2}\,a_i$), area under the curve (AUC, AUC=$\sum A_i/a_i$) and clearance (CL, CL=dose/AUC) were calculated.

Statistical analysis

All statistical comparisons were performed using Sigmastat 2.03 and Sigmaplot 5.0 was used for graphical presentations.

As pharmacokinetic data are known to be log-normally distributed,[6] geometric mean ($mean_{geom}$), geometric standard deviation (SD_{geom}) and geometric coefficient of variation (CV_{geom}) were used for descriptive statistics.[7]

Outliers of the unbound fraction of Eto (f_u) were detected according to the criteria of the '4-σ-range'[7] (value to be tested outside the range mean $\pm$ 4 SD calculated for the remaining data set: normally distributed data include 99.99%, data with any distribution 94% within this '4-σ-range'). To test for differences of f_u for total Eto $<$50, 50–100 and $>$100 mg/l, the Kruskal–Wallis test and all pairwise multiple comparison procedures (Dunn's method) were performed. To test for differences of CL for protocols administered, one-way analysis of variance for log-transformed data was performed. The relationship between normalized CL and age was evaluated by Spearman rank-order correlation.

Results

Pharmacokinetics after HD Eto

Figure 1 shows total Eto plasma concentration relative to time (data normalized to a dose of 40 mg/kg, without correction for time of infusion,

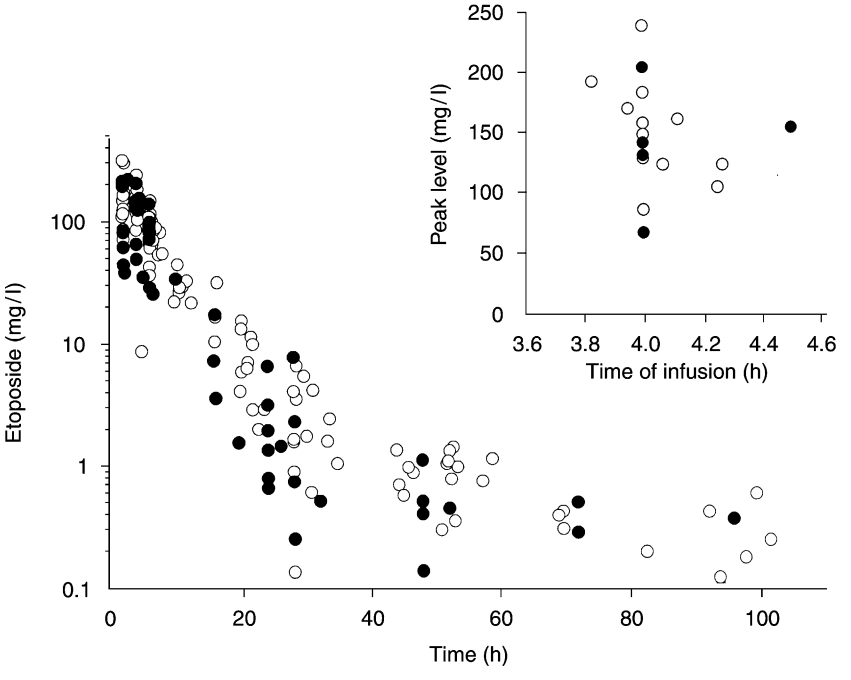

Figure 1. Concentration–time curve of total Eto (normalized to a dose of 40 mg/kg, without correction of time of infusion). Inserts: normalized peak levels as a function of time of infusion (open circles: group A, closed circles: group B).

for graphic representation) and measured normalized peak levels as a function of infusion time.

Pharmacokinetic analysis revealed that the data of group A were best fitted by a three-compartment model (model producing the smallest AIC, the best fit of the data points, and the lowest coefficient of variation of parameter estimates). The relative residual distribution showed that the error variance was best described by a heteroscedastic model (proportional to the square value of the predictions). The analysis of data from both group A and the 'intention to treat' group B led to a slight increase in coefficients of variation of almost all primary parameters, except for the variation of k_{31} which decreased slightly (Table 2). The relationship between predicted and observed concentrations of the final model is shown in Figure 2. k_{13} and k_{31} were the parameters that exhibited the highest coefficients of variation (46 and 80%, respectively); model validation indicated that this high standard error of the parameter estimates was not due to an inappropriate and overparameterized model, but that there was only little information available in the data to adequately estimate the parameter values of the deep compartment.

Derived pharmacokinetic parameters were calculated from the results for groups A and B. Half-lives, CL and AUC (normalized to 40 mg/kg) as well as the fraction of elimination associated with the first, second and third exponential term are summarized in Table 3.

The actually applied doses for all patients were calculated on the basis of body weight [40 mg/kg (20–41.9 mg/kg)] as well as body surface area

Table 2. Final population parameters for group A and groups A+B, respectively (mean ± CV)

	V_c (l/kg)	k_{12} (1/h)	k_{21} (1/h)	k_{13} (1/h)	k_{31} (1/h)	k_{10} (1/h)
Group A	0.061 ± 21%	1.263 ± 18%	0.820 ± 19%	0.038 ± 25%	0.019 ± 85%	0.526 ± 8%
Group A + B	0.066 ± 27%	1.278 ± 24%	0.886 ± 20%	0.030 ± 46%	0.019 ± 80%	0.527 ± 16%

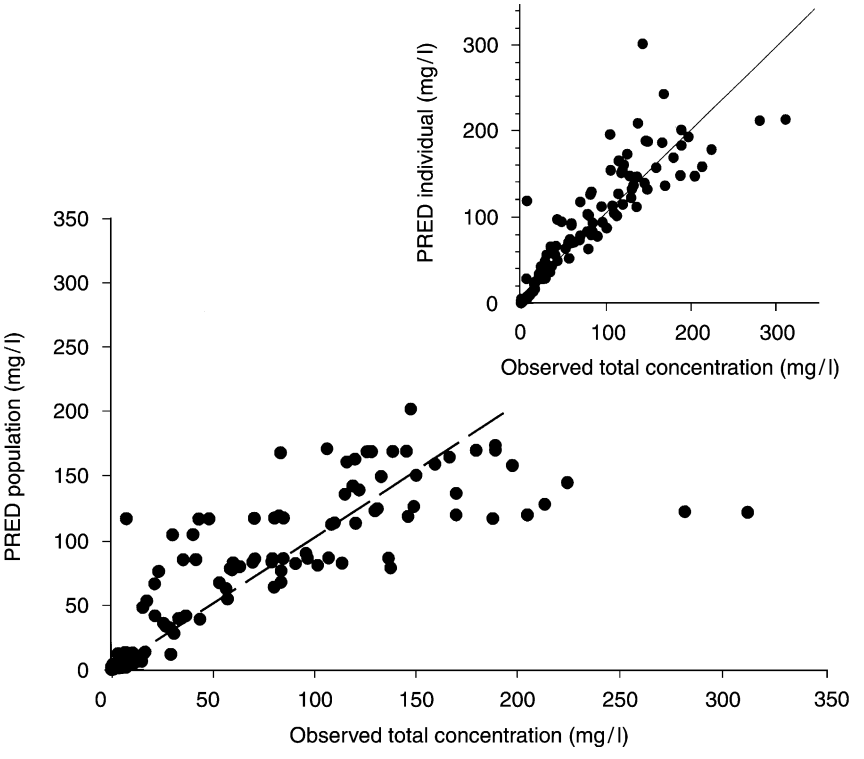

Figure 2. Relationship between concentrations predicted according to the final population model (PRED) and observed concentrations. The line of identity (dashed line) is shown. Inserts: relationship and regression line (solid line, $r = 0.924$) between individual concentrations obtained with Bayesian pharmacokinetic analysis (IPRED) and concentrations observed.

Table 3. Derived pharmacokinetic parameters for groups A+B

$t_{1/2\alpha}$[a] (h)	$t_{1/2\beta}$[a] (h)	$t_{1/2\gamma}$[a] (h)	CL[a] (ml/min/m^2)	AUC[a] (mg/l · h)	f_1[b]	f_2[b]	f_3[b]
0.27 ± 3.2%	3.6 ± 16.9%	44.2 ± 56.5%	15.5 ± 30.6%	1175 ± 27.0%	0.15 ± 9.9%	0.79 ± 2.9%	0.06 ± 28.8%

AUC calculated for a uniform dose of 40 mg/kg.
[a]Mean$_{geom}$ ± CV$_{geom}$.
[b]Mean ± CV.

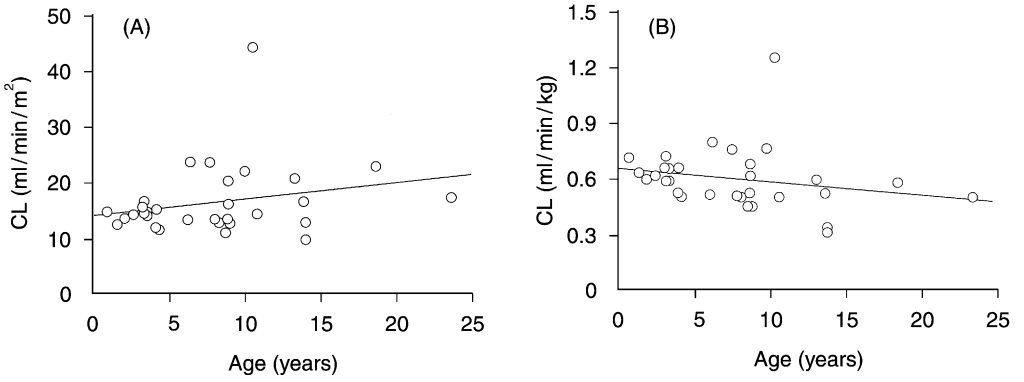

Figure 3. Relationship between CL [dose normalized: A, to body surface area (p_S = 0.31); B, to weight (p_S = 0.01)] and age of the patients (p_S: Spearman correlation coefficient).

[1000 mg/m^2 (480–1611 mg/m^2)] [median (range)]. There was no correlation between CL, dose normalized for body surface area, and age, whereas a significant correlation between CL, dose normalized for body weight, and age of the patient was found (Spearman correlation coefficient: p_S=0.31 and p_S=0.01, respectively) (Figure 3).

Unbound fraction of Eto

Total Eto plasma concentrations as well as the unbound fraction of Eto were measured. High variability was found for f_u (n=128, median 6.4%, range 1.5–57.1%). Seven values for f_u were detected as outliers (values from 22 to 57.1%; f_u without outliers: n=121, median 6.3%, range 1.5–17.6%). Outliers occurred in different protocols, five outliers were found during infusion, two outliers after the end of infusion. Patient 1 was the only one who had two of two outliers, all other patients had outliers as well as data in the normal range of f_u.

The relationship between protein binding and total Eto was examined for all data of the groups <50, 50–100 and >100 mg/l (Figure 4). Statistical comparison showed a significantly higher f_u with a total Eto >100 mg/l (median: 8.8%) as compared to the two other groups [Kruskal–Wallis test: $p < 0.001$; f_u (total Eto <50 mg/l): median 5.5%, $p_{Dunn} < 0.01$; f_u (total Eto 50–100 mg/l): median 6.5%, $p_{Dunn} < 0.05$]. Calculations without outliers showed a significant difference in f_u between total Eto >100 mg/l (median: 8.2%) and <50 mg/l (median: 5.5%) (Kruskal–Wallis test: $p < 0.001$, $p_{Dunn} < 0.01$), respectively.

As protein binding decreased for higher total Eto, intra- and inter-patient variability of protein binding was calculated based on all data as well as for the individual groups with total Eto of <100 and >100 mg/l, respectively.

The intra-patient coefficient of variation of protein binding showed high variability [all data: n=26: median 38% (range 15–76%), without outliers: n=24, median 35% (range 15–72%)]. There was only a small decrease in intra-individual variability of f_u when looking at the two groups (total Eto <100 versus >100 mg/l) [calculated without outliers: (total Eto <100 mg/l): n=18: median 25% (range 13–63%); (total Eto >100 mg/l): n=5: median 54% (range 15–60%)].

Protein binding of Eto showed high inter-individual variability [all data: n = 31, median 7.1% (range 4.3–42.2%), without outliers: median 7.0% (range 4.3–11.9%)]. Median and maximum of f_u were lower in the total Eto <100 mg/l than in the total Eto >100 mg/l group [without outliers: f_u (total Eto

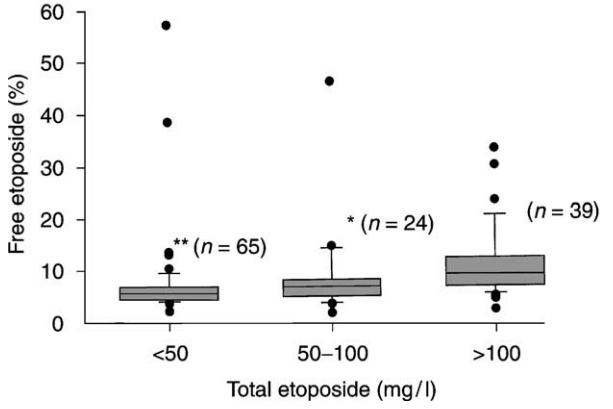

Figure 4. Free Eto as a function of total Eto. Boxes show 25th to 75th percentiles with median, whiskers indicate the 10th and 90th percentiles, outlying points are depicted as circles [Kruskall–Wallis Test: $p < 0.001$, $^{*}p_{Dunn} < 0.05$, $^{**}p_{Dunn} < 0.01$ for pairwise comparison versus f_u (total Eto >100 mg/l)].

<100 mg/l): 5.4% (range 2.7–10.9%); f_u (total Eto >100 mg/l): median 8.9% (range 2.0–17.6%)].

Discussion

Many studies have been performed to characterize the pharmacokinetics of Eto, but there are only limited data concerning the kinetics in children after HD Eto. The present study focuses on two major points of interest: data are best described by a three-compartment model and the CL of 15.5 ml/min/m^2 is at the lower range of data reported in literature.

Pharmacokinetic model

Most of the studies on the pharmacokinetics of Eto with different i.v. schedules (LD or HD, children or adults) showed that Eto disappearance from the plasma is biexponential, with mean terminal half-lives ranging from 2.2 to 9.9 h, median 5.6 h and is not dose-related (summarized in Würthwein and Boos[1] and Henwood and Brogden[8]). Data from the present drug monitoring, however, were adequately described by a three-compartment model with half-lives of $t_{1/2\alpha}=0.28\,h \pm 3.2\%$, $t_{1/2\beta}=3.6\,h \pm 16.9\%$ and $t_{1/2\gamma}=44.2\,h \pm 56.5\%$, respectively. There are only few studies in the literature[9–12] describing the decline of Eto plasma concentrations by a three-compartment model; all of these studies referred to HD in adults. In order to define factors of relevance for an adequate pharmacokinetic model after HD Eto, we reviewed appropriate pharmacokinetic studies (Table 4[2,9–19]). Doses administered ranged from 320–3500 mg/m^2, the time of infusion varied from 0.5 to 24 h; the limit of quantification of the analytical method was between 0.1 and 0.6 mg/l. None of these parameters was appropriate to distinguish studies using two- or three-compartment models, respectively. The time of sample collection after the end of infusion was the only factor clearly differentiating two groups: all studies with sample collection up to 60 h used a two-compartment model, studies with sample collection beyond 95 h resulted in a three-compartment model with a long mean terminal half-life of 17.5–44.2 h. Areas underlying each exponential term of the present drug monitoring indicate that the majority of elimination is clearly associated with the β-phase (80%) which has a half-life of 3.6 h and is thus comparable with the 'terminal β-phase' of the two-compartment models reported in literature. The area underlying the terminal γ-phase of the present data set contributes only 6%, which is in good agreement with results found by Mross *et al.*[11] (7 ± 5% of area was associated with the terminal γ-phase).

Eto clearance

CL data presented here [15.5 ml/min/m$^2 \pm 30.6$% (mean$_{geom} \pm$ CV$_{geom}$)] suggest the possibility of lower CL values for HD Eto in children as compared to HD Eto in adults [21.8 ml/min/m^2 (17.7–47.4 ml/min/m^2)], whereas Eto pharmacokinetic studies in children at lower doses [CL: 21.0 ml/min/m^2 (17.4–39.6 ml/min/m^2)] do not provide any suggestion of such differences (median and range of mean values reported in the literature, summarized in Würthwein *et al.*[1]) (Figure 5).

The relatively low Eto CL together with findings of a deep third-compartment detected under different conditioning regimens may not only influence AUC but also plasma drug concentrations at the time of BMT. Clinical and *in vitro* data suggest that BMT should be delayed until the plasma Eto concentrations have fallen to less than 0.4 mg/l.[20,21] Therefore, we compared our findings with the recently introduced simulation tool for Eto. Based on kinetic findings after LD Eto in children, the tool showed good predictability for LD and HD schedules in terms of total drug exposure and in terms of concentration–time profiles reported in the literature. Limitations pointed out concerned co-administration of other agents that are known to influence Eto CL significantly or renal impairment. We calculated

Table 4. Half-lives (mean ± CV) after HD Eto reported in the literature

Author	No. of patients	Dose (mg/m^2)	Time of sample collection after end of infusion (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)
Kreis 1996[13]	3	750	22	3.0 ± 24.4%	5.2 ± 20.8%	
Kreis 1996[13]	6	1000	22	2.2 ± 12.6%	7.3 ± 17.3%	
Kreis 1996[13]	4	1200	22	2.1 ± 23.7%	4.7 ± 48.3%	
Köhl 1992[14]	10/27 cycles	700	23.5	0.4 ± 19.2%	4.3 ± 14.8%	
Hande 1984[15]	12	400–800	24	0.8[a]	8.1[a]	
Rodman 1994[2]	29	320–500	24		5.9[b] ± 90.0%	
Slevin 1989[16]	20	500	24		7.4 ± 17.6%	
Steward 1984[17]	35/113 cycles	600	24	0.3 ± 12.1%	6.1 ± 8.2%	
Newmann 1988[18]	13	1000–3000	56		4.3 ± 25.6%	
Schwinghammer 1993[19]	16	35–60 mg/kg	60	0.7 ± 129%	7.2 ± 51.4%	
Present study	31	40 mg/kg (1063)	97	0.3[c] ± 3.2%	3.6[c] ± 16.9%	44.2[c] ± 56.5%
Mross 1994 (BMT)[11]	9	30–45 mg/kg	102	0.5 ± 100%	3.0 ± 20.0%	20.1 ± 63.7%
Mross 1994 (JCO)[10]	17	30–45 mg/kg	120	0.4 ± 108%	3.3 ± 44.1%	17.5 ± 69.2%
Mross 1996[12]	7	30–45 mg/kg	120			20.4 ± 69.6%
Mross 1996[12]	6	30–45 mg/kg	144			33.7 ± 26.4%
Holthuis 1986[9]	12/72 cycles	500–3500	180	2.7 ± 49.4%	23.2 ± 113%	($\gamma = 0.68 \times 10$–4)

[a]Fitted to post-infusion data.
[b]Median.
[c]Mean$_{geom}$ ± CV$_{geom}$.

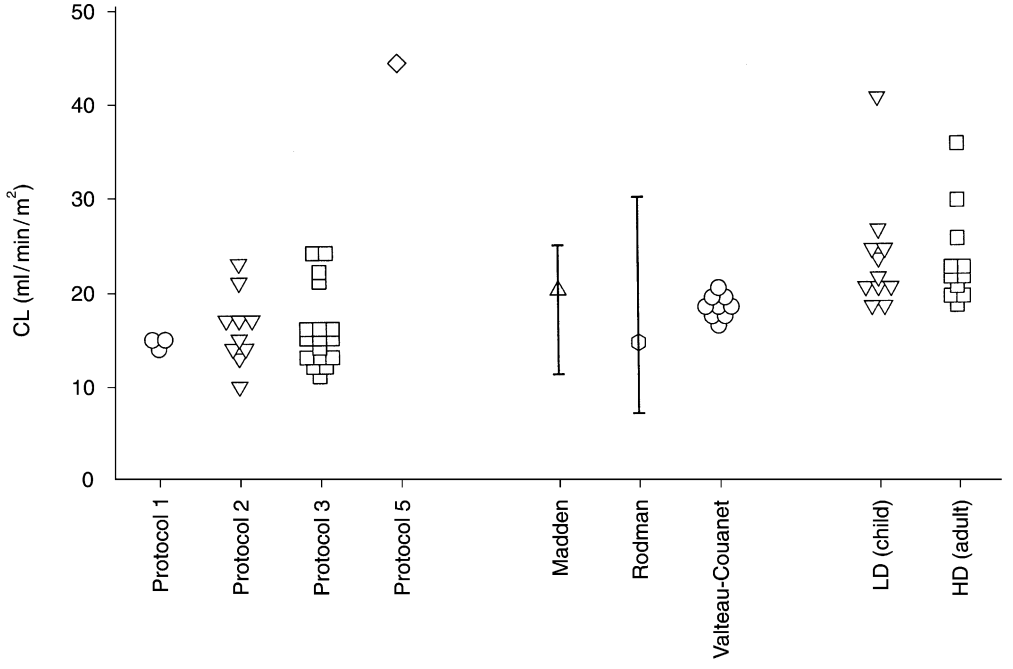

Figure 5. CL of the present study as a function of protocol together with CL reported in literature. HD in children: Madden *et al.*[4] and Rodman *et al.*[2]: median, range, Valteau-Couanet *et al.*[3] Data from the literature after LD in children and HD in adults: mean values reported in the literature, summarized in Würthwein *et al.*[1].

concentration-time profiles for 1000 mg/m^2/4 h based on the present population and based on the simulation tool (Figure 6). The present data set showed a 50% higher AUC (1075 ± 30.6 versus 721 mg/l · h ± 20.0%, mean$_{geom}$ ± CV$_{geom}$) as well as a 2-fold increase in the duration of Eto concentrations above 0.4 mg/l (62.2 ± 20.1 versus 33.2 ± 7.0 h, mean ± SD) over the values predicted by the

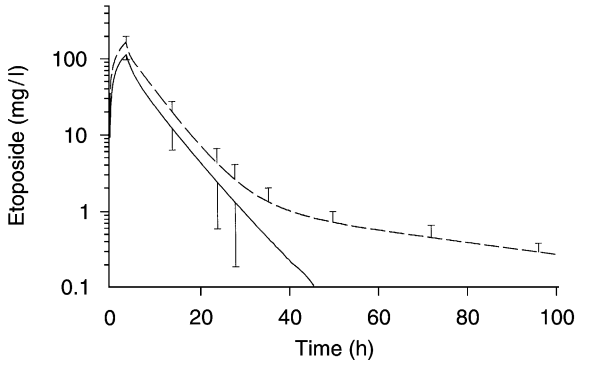

Figure 6. Concentration–time curve of Eto after a 4-h infusion of 1000 mg/m^2. Simulations based on (solid line) Würthwein *et al.*[1] and (dashed line) data from the present study (mean ± SD).

simulation tool. However, Eto concentrations at the time of BMT—96 or 144 h after Eto administration—were predicted to be below 0.4 mg/l in all patients.

Only few studies have been published on the kinetics of Eto in children at higher doses (Figure 5): Madden *et al.*[4] administered an average dose of 2000 mg/m^2 as a 96-h infusion and found a median CL of 19.9 ml/min/m^2 (range 11.0–24.6 ml/min/m^2). Valteau-Couanet *et al.*[3] treated nine children with 1800–2400 mg/m^2 as a 72-h continuous i.v. infusion, CL was 17.6 ml/min/m^2 (range 15.7–19.6 ml/min/m^2). Rodman *et al.*[2] treated 29 children at doses of 960–1500 mg/m^2 in three divided doses as a 6-h infusion on alternate days with HD carboplatin. The median Eto CL in all patients of this study was 14.3 ml/min/m^2 (range 6.8–29.6 ml/min/m^2) and was 13.4 ml/min/m^2 (range 6.8–29.6 ml/min/m^2) in those 22 patients who did not receive concurrent anticonvulsant therapy, which is known to increase Eto CL. Rodman *et al.* discussed the co-administration of HD carboplatin to impair Eto metabolism.

In order to determine the potential effect of co-administrations in the present drug monitoring we calculated Eto CL for the different conditioning regimens: Eto CL in one patient treated according to protocol 5 was 44.1 ml/min/m^2. Co-administration of phenobarbital, known to induce cytochrome P-450 enzymes, may explain this high CL value. Patients treated according to protocol 3 showed low Eto CL of 15.0 ml/min/m^2 ± 25.1% (mean$_{geom}$ ± CV$_{geom}$). In this protocol, co-administration of HD carboplatin was part of the conditioning regimen. Patients treated according to protocols 1 or 2, without co-administration of carboplatin, showed CL values which were not significantly different from CL values calculated for protocol 3 [protocol 1: 14.3 ml/min/m^2 ± 4.3%; protocol 2: 15.1 ml/min/m^2 ± 25.3% (mean$_{geom}$ ± CV$_{geom}$), one-way analysis of variance for log-transformed data: $p = 0.91$]. Most patients treated according to these two protocols received CSA, which might be responsible for reduced Eto CL.[22] Are co-administrations of carboplatin or CSA responsible for the low Eto CL or is HD Eto treatment in children generally associated with lower CL values than seen in adults: based on the present rather heterogeneous population this question remains open. Further clarification might be possible by prospective drug monitoring, comparing CL after HD Eto in children and adults.

Eto CL related to body surface area or body weight

Eto CL as related to body surface area was found to be unrelated to patient age, whereas CL as related to body weight decreased with increasing age of the patients. However, due to the high inter-individual variability in Eto CL, the significant correlation found between CL/kg and age should be discussed with caution. For LD Eto (173 treatment courses in 78 children, 96 h continuous infusion, 125 mg/m^2) Boos *et al.*[5] showed that both CL related to body surface area as well as CL related to body weight were independent of age. Eksborg *et al.*[23] (16 children, 1–3-h infusion, 32–210 mg/m^2) examined AUC: AUC, dose normalized to body surface area, was independent of age, while AUC normalized to dose in mg/kg, increased with increasing age of the patients. Thus, under LD as well as under HD Eto, CL/m^2 was independent of age, while the relationship between CL/kg and age cannot be clarified definitively.

Unbound fraction of Eto

A high range of f_u was measured, with seven of 128 (22.1–57.1%) data found as outliers. Preanalytical errors (five of seven outliers were measured during infusion; thus a contamination while taking blood samples from the infusion tube might be possible), analytical errors and physiological influences (patient 1 showed two of two data with high f_u, 38.5 and 46%) might be responsible for these high values of f_u. As extreme values were found for all protocols included in the drug monitoring, the influence of different co-administrations cannot be addressed.

In the present study we found an increase in f_u for total Eto > 100 mg/l compared to f_u for total

Eto < 100 mg/l. A comparable study in adults[19] (16 patients, 35–60 mg/kg, 4-h infusion) undergoing autologous BMT for advanced lymphoma, showed that the percentage of protein binding was significantly higher at the end of infusion (total Eto: 120 mg/l ± 47, f_u = 21%) than at the lowest measured concentration (f_u = 13%; high values of f_u were found due to hypoalbuminemia). Studies after LD Eto[24,25] showed no such dose dependency, suggesting that some concentration dependent variability in binding may occur only at high Eto levels.

We saw high intra- and inter-patient variability in the protein binding of Eto [(35% (15–72%); 7% (4.3–11.9%), data without outliers, median (range)], which is in good agreement with data reported after LD[26–30] or HD[19] Eto.

In summary, the Eto concentration–time profile after HD in children was best described by a three-compartment model. Eto CL was low compared to previously published data. High inter- and intra-individual variability of protein binding was found with an increase of f_u at high total Eto concentrations.

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