Doxorubicin pharmacokinetics is correlated to the effect of induction therapy in children with acute myeloid leukemia

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We studied the pharmacokinetics of doxorubicin in 41 children treated for newly diagnosed acute myeloid leukemia. Doxorubicin, 75 mg/m^2 body surface area, was administered by constant i.v. infusion over 8 h. Four children with Down's syndrome (DS), 1.2–2.3 years old, had a median total body clearance of 523 ml/min/m^2. The median clearance in non-DS children, 0.6–1.8 years old ($n=4$) and 2.5–17.7 years old ($n=33$), was 446 and 538 ml/min/m^2, respectively. Patients who went into complete remission (CR) after induction therapy had a significantly higher median plasma concentration of doxorubicin than those who did not, 249 compared with 180 ng/ml, respectively ($P=0.036$; analysis restricted to non-DS patients). Doxorubicin plasma concentration was an independent factor for CR, both in univariate ($P=0.031$) and multivariate analysis including sex, age and white blood cell count at diagnosis ($P=0.021$). Patients who reached CR had a significantly lower doxorubicin clearance than those who did not, 513 and 657 ml/min/m^2, respectively ($P=0.017$). In conclusion, doxorubicin plasma concentration and total body clearance during up-front treatment were correlated to the effect of induction therapy.

Prospective studies should be performed to confirm the concentration–effect relationship and explore the possibility of therapeutic monitoring. *Anti-Cancer Drugs* 17:385–392 © 2006 Lippincott Williams & Wilkins.

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Introduction

The anthracycline doxorubicin is widely used in the treatment of malignant disease, including leukemias and solid tumors [1,2]. In adults it has been shown that the pharmacokinetics of the drug are highly variable, with an almost 10-fold inter-patient variation of the area under the plasma concentration–time curve (AUC), despite standardization of the dose based on body surface area (BSA) [2–5]. The reason for the high variability of doxorubicin pharmacokinetics is largely unknown, but it has been suggested that there might be a need for dose reduction in patients with elevated serum aminotransferases and/or serum bilirubin [3]. Only scant information is available concerning pharmacokinetic interaction of the anthracyclines with other anti-neoplastic drugs [2]. Side-effects of doxorubicin include nausea/vomiting, mucositis, myelosuppression and cardiotoxicity. The risk for cardiomyopathy is related to the cumulative dose [6], but there also appear to be differences in sensitivity between individuals [7]. The considerable inter-individual differences in doxorubicin pharmacokinetics, together with

unpredictable variability in toxicity and clinical outcome, has led to a discussion of whether doxorubicin might be a candidate for therapeutic drug monitoring [8,9].

Previous publications on the pharmacokinetics of doxorubicin in children are relatively few [10–16] and a number of points still remain to be clarified. Only limited data are available for infants – an age group where the dosing of drugs often is a problem [17]. 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 anti-cancer drugs [17–19]. This has generally been related to pharmacodynamics, but it might also be due to differences in drug distribution or elimination. No data on doxorubicin pharmacokinetics in DS children have been published. The most striking lack of knowledge, however, concerns the correlation between dose, plasma concentration and clinical outcome. In adult patients, a correlation between plasma concentrations of doxorubicin and the outcome

of remission induction therapy has been reported [20], but such relationships have not been demonstrated in childhood malignancies (see [21] for a review).

The aim of the present investigation was to study the pharmacokinetics of doxorubicin and its active metabolite doxorubicinol in children with AML, treated according to a common protocol at Nordic centers for pediatric oncology. The maximum plasma concentration, reached at the end of a constant infusion, can be used as a substitute for measuring the AUC. This simplified technique of pharmacokinetic monitoring, which is well documented for the anthracyclines [13,14], was used in the present study. Pharmacokinetic data were correlated to clinical effect, estimated both by bone marrow morphology after remission induction therapy and by long-term clinical follow-up.

Methods
Patients
Between March 1995 and September 2000, 41 children were included in the study at eight Nordic centers for pediatric oncology: Copenhagen, Gothenburg, Helsinki, Linköping, Lund, Tampere, Oslo (Ullevål) and Uppsala. Four of the children had DS. As expected, the DS children were younger than the non-DS children and also differed in their distribution of FAB types, for details see Table 1. Since 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 Pediatric Hematology and Oncology (NOPHO) AML-93 protocol [22] and studied during the first induction course. As shown in Fig. 1, this course included an intrathecal injection of methotrexate, usually administered when the child was under anesthesia to establish a central venous line for administration of drugs and a peripheral venous catheter for blood sampling. Etoposide, 100 mg/m^2 BSA/24 h and cytarabine, 200 mg/m^2

BSA/24 h, were administered concomitantly by constant infusion pump over a 96-h period on days 1–4. 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. anti-emetics, 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 or older and $m^2 = \text{weight (kg)}/30$ for children under 2 years old. Thus, infants received 2.5 mg doxorubicin/kg body weight.

According to the protocol, a bone marrow sample was drawn 3 weeks after the start of the induction course (median 24 days, range 13–40 days) to evaluate treatment response. Less than 5% blast cells in a stained smear of a non-hypoplastic 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 hemopoiesis or regrowth of malignant cells emerged. Twenty-eight out of the 41 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 13 of the 28 patients receiving 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 consolidation blocks. The backbone of this treatment was high-dose cytarabine, administered as a single drug (one course), or combined with etoposide (two courses) or mitoxantrone (one course). Children with a matched related donor were candidates for allogeneic stem cell transplantation (SCT) in first remission.

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.

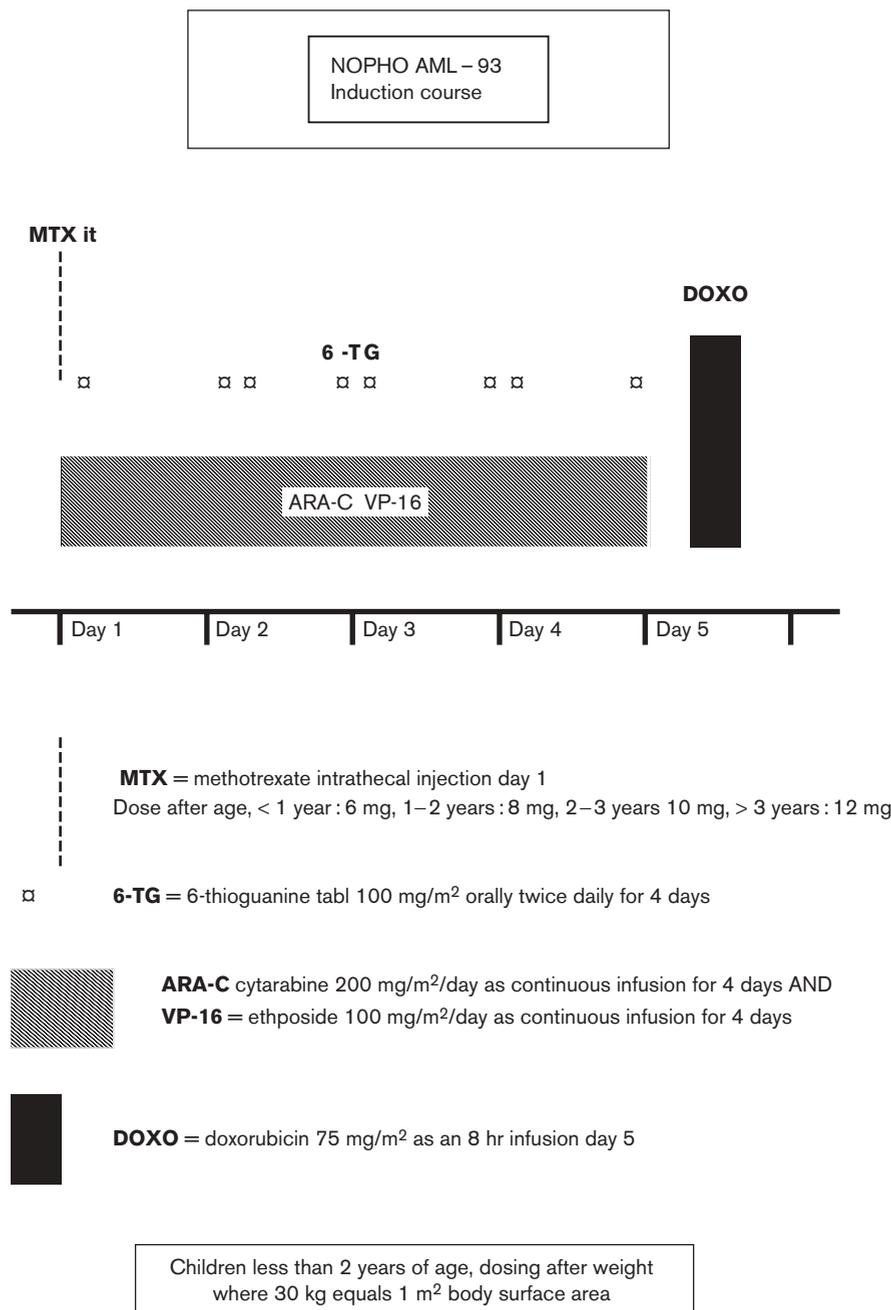Local ethics committees approved the study.

Plasma samples
A blood sample was drawn 7 h after start of the doxorubicin infusion, i.e. 1 h before the infusion was completed. Blood was drawn from a venous line not used for doxorubicin infusion and collected in tubes containing EDTA.

Patient data (body weight, height, actual dose administered), as well as exact times for start and stop of

Table 1 Patient characteristics

	Non-DS	DS	P
Number	37	4	
Age (years)			
median	9.2	1.9	0.009
range	0.63–17.7	1.2–2.3	
Sex (n)			
male	16	1	
female	21	3	
WBC (10^9/l)			
median	9.7	26.2	0.50
range	0.6–190	7.1–44.7	
FAB (n)			
M0	1	0	
M1	8	2	
M2	11	0	
M3	1	0	
M4	8	0	
M5	6	0	
M7	1	2	
other	1	0	

Fig. 1

Induction course.

Children less than 2 years of age, dosing after weight where 30 kg equals 1 m² body surface area

DOXO = doxorubicin 75 mg/m² as an 8 hr infusion day 5

ARA-C cytarabine 200 mg/m²/day as continuous infusion for 4 days AND
VP-16 = ethposide 100 mg/m²/day as continuous infusion for 4 days

6-TG = 6-thioguanine tabl 100 mg/m² orally twice daily for 4 days

MTX = methotrexate intrathecal injection day 1
Dose after age, < 1 year : 6 mg, 1–2 years : 8 mg, 2–3 years 10 mg, > 3 years : 12 mg

infusions, and for blood sampling, were noted. Serum concentrations of creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), determined before the start of the induction course, were also recorded.

Analytical procedure
Plasma concentrations of doxorubicin and doxorubicinol were determined by reverse-phase isocratic HPLC adopted and modified from Paul *et al.* [23]. In brief, 100 µl of idarubicin (10 µmol/l) was added to 1 ml plasma. An aliquot of 500 µl of 0.1 mol/l borate buffer (pH 9.3) was added and extraction was performed after addition of 6 ml chloroform : methanol (4 : 1 v/v). The chloroform phase was collected and evaporated to dryness. Thereafter, the residue was reconstituted by the addition of 200 µl of mobile phase (0.2% ammonium formiate pH 4/ acetonitrile, 73 : 27). Then, 50 µl was injected, and doxorubicin, doxorubicinol and the internal standard

idarubicin were separated on a micro-Bondapack phenyl column at a flow rate of 1.5 ml/min. Quantitation was made by fluorescence (excitation/emission wavelengths 520/600 nm).

Pharmacokinetic evaluation and statistics

Based on recorded data for body weight and height we recalculated all patients' BSA by the formula $m^2 = \sqrt{[height\ (cm) \times weight\ (kg)/3600]}$. 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 steady-state concentration of the drug. It has been shown that 80% of the steady-state concentration of doxorubicin is reached after 7 h of infusion [4,14,24] and this was compensated for in the calculations of plasma clearance by dividing the observed 7-h doxorubicin concentration by 0.8.

The Spearman rank-test (two-sided) was used to examine correlations, the Mann–Whitney U-test to compare values from two groups and logistic regression analysis to test the probability of a defined event including one (univariate) or several covariates (multivariate analyses). The SPSS 12.0 software package (SPSS, Chicago, Illinois, USA) was used for the calculations. $P < 0.05$ was considered as statistically significant.

Results

The median doxorubicin dose received by non-DS children 2 years of age or older (range 2.5–17.7 years) was 74.9 mg/m², which was very close to the target dose of 75 mg/m². The median dose received by children under 2 years of age (range 0.6–1.8 years) was 61.7 mg/m² ($P = 0.013$; Table 2).

The median doxorubicin concentration in plasma was 232 and 238 ng/ml in children above and below 2 years of age, respectively. As evident from Table 2, there was a large inter-individual variation in plasma levels. Median total body clearance was 538 ml/min/m² in children aged 2 years or older and 446 ml/min/m² in children below 2 years of age (NS). The four infants aged 0.6, 1.0, 1.3 and 1.8 years had clearance values of 298, 590, 1128 and 303 ml/min/m², respectively.

The median plasma concentration of the metabolite doxorubicinol was 29 and 41 ng/ml in children above and below 2 years of age, respectively (NS). Median values for doxorubicinol calculated as percent of doxorubicin were 17 and 29% in these groups (NS). Higher concentrations of doxorubicinol were found in patients with high doxorubicin levels ($\rho = 0.38$; $P = 0.024$).

Total body clearance of doxorubicin was used to explore the correlation between pharmacokinetics and background variables, and all non-DS children were included in this analysis. Boys had a higher clearance than girls, 591 and 427 ml/min/m², respectively ($P = 0.020$), while age, weight, height, ALT, AST, creatinine, albumin, white blood cell (WBC) count at diagnosis and dosage (mg/m²) were non-significant. Most children had normal or near normal ALT, AST, albumin and creatinine values. Two children with elevated ALT values, here defined as levels above 2 times normal, had clearance values of 593 and 465 ml/min/m². There was no correlation between doxorubicinol concentrations and any of the background variables mentioned above.

Children with DS

Four children with DS were studied, 1.2, 1.8, 1.9, and 2.3 years old, respectively. They received a median doxorubicin dose of 42.5 mg/m² (Table 2). The median doxorubicin clearance of the DS children was 523 ml/min/m² – a value comparable to that of non-DS children. The median doxorubicin concentration in plasma was 151 ng/ml, which represented 65% of that found in non-DS children ($P = 0.14$).

Table 2 Summary of pharmacokinetic parameters in children with or without DS

	Non-DS <2 years	P^a	Non-DS >2 years	P^b	DS
Number	4		33		4
Dose (mg/m²)					
median	61.7	(0.013)	74.9	(0.002)	42.5
range	55.0–74.6		61.7–79.0		26.7–63.3
Plasma concentration (ng/ml)					
median	238	(0.90)	232	(0.14)	151
range	110–348		29–510		87–238
Clearance (ml/min/m²)					
median	446	(0.53)	538	(0. 48)	523
range	298–1128		254–4211		348–551
Doxorubicinol concentration (ng/ml)					
median	41	(0.50)	29	(0.56)	29
range	29–110		29–116		29–52
Dox-ol% (doxorubicinol concentration/doxorubicin concentration × 100)					
median	29	(0.21)	17	(0.42)	24
range	8–36		7–45		12–47

[a]For <2 compared with >2-year-old non-DS children.
[b]For DS compared with all non-DS children.
Where not otherwise stated, data refer to doxorubicin.

The median doxorubicinol plasma level was 29 ng/ml, corresponding to 24% of the doxorubicin concentration.

## Pharmacokinetics compared with effect in non-DS children

Twenty-six out of 37 patients (70%) went into CR after the first treatment course, whereas 11 did not. Patients who reached CR had a significantly higher median plasma concentration of doxorubicin than those who did not, 249 (178–326) and 180 ng/ml (25th–75th percentiles 113–226), respectively ($P = 0.036$; Fig. 2). Figure 3 shows the predicted probability of CR as a function of doxorubicin concentration in a univariate analysis, indicating a concentration–effect relationship ($P = 0.031$). In multivariate analysis including sex, age and WBC count at diagnosis, doxorubicin concentration was the only independent factor for CR ($P = 0.021$), with a trend value for age ($P = 0.082$; less probability for CR with increasing age). Natural-log transformation of doxorubicin concentrations gave similar results, with $P = 0.029$ in univariate and $P = 0.018$ in multivariate analysis (not shown). Patients who reached CR had a significantly lower median doxorubicin clearance than those who did not, 513 (364–603) and 657 ml/min/m^2 (25th–75th percentiles 538–1488), respectively ($P = 0.017$). There was no difference in doxorubicinol concentrations between the groups ($P = 0.14$).

Fig. 2
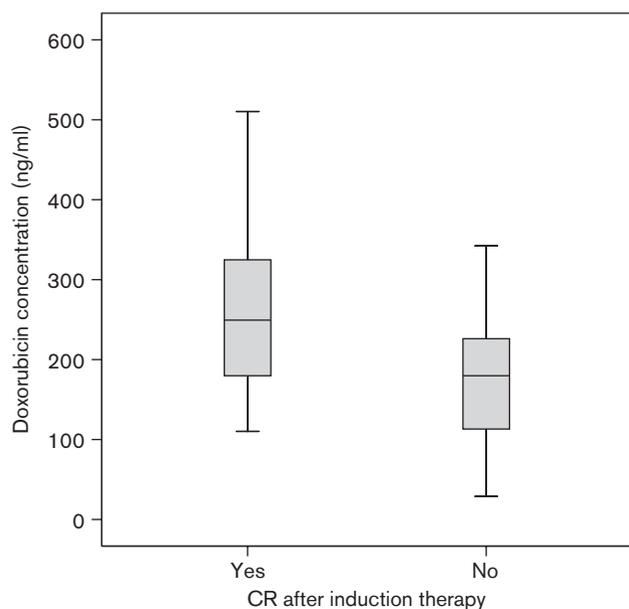
Doxorubicin plasma concentration in children who went into CR after the first induction course ($n=26$) and those who did not ($n=11$). Children with DS are excluded. The box-and-whisker plot shows median, first and third quartiles; whiskers extend to the highest and lowest value.

Fig. 3
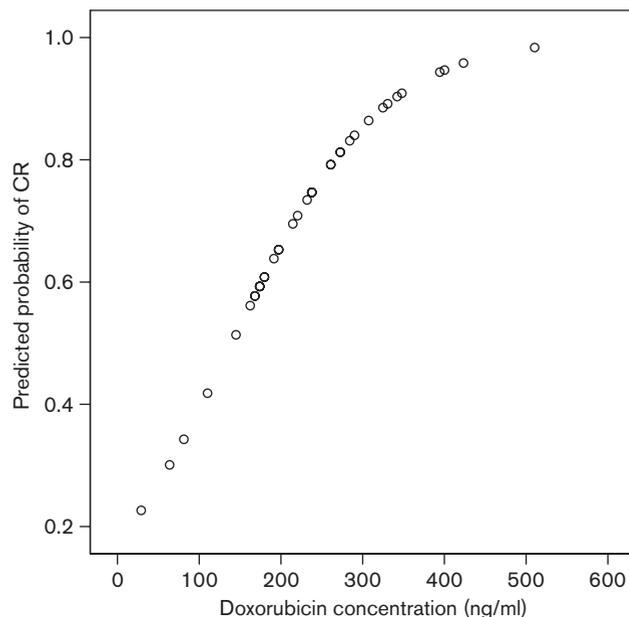

Predicted probability of CR after induction therapy as a function of doxorubicin concentration ($n=37$). Children with DS are excluded.

Twenty patients were in continuous complete remission (CCR) at the latest follow-up (nine after allogeneic SCT in the first CR), while 15 had relapsed (four after allogeneic SCT in the first CR), with a median follow-up time of 7.3 years (range 4.3–9.7 years). Two patients died in CR after allogeneic SCT in the first CR. Median values for doxorubicin plasma levels were 238 and 191 ng/ml ($P = 0.22$) and total body clearance 517 and 636 ml/min/m^2 ($P = 0.19$) in CCR and relapsed patients, respectively. Doxorubicin concentration was not an independent factor for CCR in univariate ($P = 0.14$) or multivariate analysis including SCT ($P = 0.12$). The two patients who died in CR had doxorubicin concentrations of 232 and 400 ng/ml, respectively.

Repeated courses

Repeated sampling was successful in 13 patients (one with DS) receiving a second treatment course identical to the first one. The median interval between the start of the courses was 28 days (range 25–47 days). Median clearance was 518 (439–651) and 588 ml/min/m^2 (25th–75th percentiles 415–694) for courses 1 and 2, respectively. As evident from Fig. 4, a number of patients had stable clearance values from course to course, but marked changes were also seen, both increases and decreases. The correlation between the clearance values from the two courses was not statistically significant ($\rho = 0.37$; $P = 0.21$). Median doxorubicinol as percentage of doxorubicin was 17 and 16% for the two courses ($\rho = 0.38$; $P = 0.20$).

Fig. 4
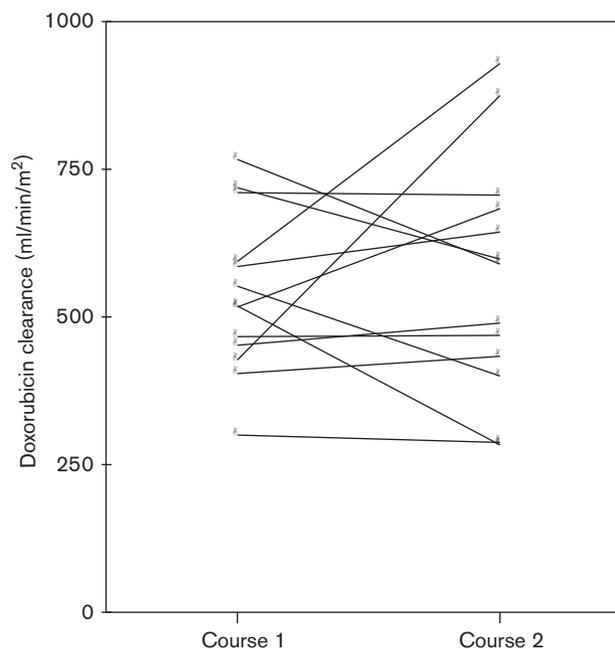
Doxorubicin total body clearance measured during identical treatment courses administered with a 4-week interval. Lines connect individual values.

Discussion

The median total body clearance of doxorubicin found here is similar to that reported for children treated for acute lymphoblastic leukemia (ALL) [15]. In the ALL study, doxorubicin was infused after injection of vincristine and during concomitant treatment with oral prednisolone. Here, doxorubicin was given on day 5 of an intense induction course, preceded by a 4-day continuous infusion of etoposide and cytarabine with concomitant administration of oral 6-thioguanine. Apparently, the pharmacokinetics of doxorubicin were not significantly influenced by any of these drugs.

In agreement with previous studies, we found a large inter-individual variation, with more than 10-fold differences in steady-state concentrations and clearance values. Correlation to known background variables could only explain these differences to a limited extent.

Published data regarding the age dependency of doxorubicin pharmacokinetics in children are equivocal. McLeod et al. [10] observed a lower clearance, normalized for BSA, in children younger than 2 years as compared to children aged 2–20 years. In contrast, Crom et al. [16] found that plasma clearance of doxorubicin, normalized to BSA, was not related to age. Eksborg et al. [13] did not find a clear correlation between age and pharmacokinetics, while Frost et al. [15] found higher steady-state

plasma levels of doxorubicin in children aged 4–6 years than in younger and older children. In the present study, we found no statistically significant correlation between pharmacokinetic parameters and age. Thus, the present study did not yield data that could improve doxorubicin dosage compared to that based on BSA.

Very little data on the pharmacokinetics of doxorubicin has been reported for individuals below 1 year of age. McLeod et al. found a trend toward a lower rate of systemic clearance based on BSA in four infants, 0.17–0.83 years old [10]. Frost et al. found that four infants, 7–9 months old, had plasma clearance between 350 and 431 ml/min/m^2, while a 3-month-old infant had a clearance of 181 ml/min/m^2. In the present study, one 0.6- and one 1.0-year old infant had clearance values of 298 and 590 ml/min/m^2, respectively. These limited data together indicate that infants above 6 months of age have plasma clearance similar to that of children over 1 year old, and that major dose reduction is not necessary from a pharmacokinetic point of view. For infants below 6 months of age, dose reduction is recommended in most protocols [16] and the single observations available are in accordance with this strategy.

In adults, a lower plasma clearance of doxorubicin has been observed in females as compared to males [25]. It has also been reported that nausea associated with anthracycline-containing regimens is more severe in girls than in boys [26] and that girls run a higher risk of abnormalities in cardiac function [27,28]. In the present study, we found that boys had higher clearance values than girls, but Frost et al. found no such difference in children treated for ALL [15]. Different doses of doxorubicin for boys and girls cannot be considered until this matter has been further explored.

Doxorubicinol is the only cytotoxic metabolite of doxorubicin [29]. The in-vitro toxicity of doxorubicinol has been reported to be similar to [30] or lower [29,31] than that of doxorubicin, but the influence of the metabolite on the therapeutic effect and the side-effects after doxorubicin administration are unknown. Some data suggest that doxorubicinol might contribute to the cardiotoxicity seen after treatment with doxorubicin [32]. Since the median doxorubicinol/doxorubicin quotient was 0.17 and these two compounds have similar half-lives [2], our findings indicate that doxorubicinol generally adds little to the therapeutic effect in children. A few patients had a considerably higher quotient, however, and in these cases doxorubicinol could be of some importance.

Children with DS have an increased risk of developing acute leukemia, especially AML, where 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 [22,33–37]. However, there is still considerable uncertainty about the optimal dosing of drugs administered in multi-agent treatment courses, since the effect and toxicity of individual drugs are very difficult to evaluate. To our knowledge, data on doxorubicin pharmacokinetics have not been published for children with DS. The NOPHO AML-93 protocol had no recommendations for dosage of doxorubicin in DS children, but we found that the four patients studied here in practice received considerably reduced doses. The four DS children, aged 1.2–2.3 years, had a median doxorubicin clearance of $523\,\mathrm{ml/min/m^2}$, a value comparable to that of non-DS children. This indicates that dose reduction is not necessary in DS children above 1 year old from a strictly pharmacokinetic point of view. These data need to be confirmed in a larger number of patients, however, and the possibility of an increased susceptibility to the side-effects of doxorubicin in DS children must also be taken into account when treatment protocols for this group of children are discussed. Furthermore, blast cells from DS children with AML are significantly more sensitive *in vitro* to doxorubicin (and several other anti-neoplastic drugs) than cells from non-DS children with AML [38,39]. In AML, excellent results in DS patients have been reported with reduced drug intensity [34,35] and the present data are therefore no argument against the current policy to treat AML in DS children with reduced doses of anthracyclines.

A relationship between plasma concentrations of doxorubicin and the outcome of induction therapy has been reported for AML in adults [20], but to our knowledge there are no studies of the correlation between doxorubicin plasma levels and therapeutic effect in childhood leukemia or any other pediatric malignancy [21]. Here, we found a statistically significant correlation between doxorubicin pharmacokinetics and the effect of remission induction therapy. Patients who reached CR had significantly higher plasma levels and lower total body clearance of doxorubicin than those who did not. Doxorubicin concentration was an independent factor for CR in univariate ($P = 0.031$) and multivariate analysis including sex, age and WBC count at diagnosis ($P = 0.021$).

At long-term follow-up, patients who remained in CCR tended to have higher plasma levels of doxorubicin than those who relapsed, but the difference was not statistically significant. Doxorubicin concentration was not an independent factor for CCR in univariate or multivariate analysis. This discrepancy between short- and long-term clinical effects might partly be due to the fact that doxorubicin was given during induction therapy only, while the four consolidation courses consisted of cytarabine, etoposide and mitoxantrone. The fact that 41% of the non-DS patients received allogeneic SCT in first

remission is also an obvious confounding factor in the evaluation of long-term effects of doxorubicin. The number of patients treated without SCT was too small for meaningful analysis.

Even when a relationship between plasma concentrations and effect can be established, pharmacokinetically guided therapy is only feasible if there is a limited intra-individual variability from course to course. Thirteen of the children in this study who went into CR after the first induction course received a second identical course 3–4 weeks later. Although some patients showed very little course-to-course variability, the results were quite unpredictable in others.

In conclusion, doxorubicin plasma concentration and total body clearance during up-front treatment were correlated with the effect of induction therapy. The correlation between doxorubicin plasma concentrations and clinical effect should be validated in prospective studies. Course-to-course variability in doxorubicin pharmacokinetics also has to be studied further before therapeutic drug monitoring can be recommended.

Acknowledgments

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