Cancer Chemother Pharmacol (2003) 51: 465–473
DOI 10.1007/s00280-003-0602-3

ORIGINAL ARTICLE

Kamilla B. Petersen · William J. Jusko
Mette Rasmussen · Kjeld Schmiegelow

Population pharmacokinetics of prednisolone in children with acute lymphoblastic leukemia

Received: 20 November 2002 / Accepted: 12 February 2003 / Published online: 16 April 2003

Abstract *Purpose*: To evaluate the plasma protein binding and pharmacokinetics of prednisolone during therapeutic use in children with acute lymphoblastic leukemia (ALL) using the population approach. *Methods*: A two-compartment pharmacokinetic model was used to describe data from 23 children with ALL (aged 2–15 years). Prednisolone (60 mg/m^2 per day in three divided doses) was administered both orally and intravenously, and samples were obtained on several days during the initial 5 weeks of remission induction therapy. Unbound plasma concentrations ($n = 288$) were determined by HPLC and ultrafiltration. Nonlinear mixed-effects modeling (WinNonMix version 2.0.1) was used to estimate the pharmacokinetic parameters, to identify significant covariates, and to estimate the protein binding parameters. *Results*: Prednisolone showed complete oral bioavailability. The median unbound clearance (32 l/h per m^2) was lower, and the half-life (3.6 h) longer than previously reported in childhood ALL. Body weight was a significant covariate for the central and peripheral volumes of distribution resulting in interindividual variabilities of 50% and 42%. Including body surface area as a covariate for clearance decreased the interindividual variability to 14%. The estimated areas under the unbound plasma concentration-time curves showed less than twofold variation among patients, and a residual variability of 20% indicated that the pharmacokinetic parameters remained stable during induction therapy. The estimated protein binding parameters were comparable to, but slightly lower than, previously published values and independent of the albumin concentration. *Conclusions*: The study showed complete oral bioavailability, a lower unbound clearance and a longer half-life for prednisolone than previously reported in childhood ALL. Plasma protein binding was independent of the albumin concentration. Due to the small inter- and intraindividual variations in the pharmacokinetic parameters, body surface area-based dosing is sufficient to obtain similar systemic exposure among patients.

Keywords Acute lymphoblastic leukemia · Child · Population pharmacokinetics · Prednisolone

The study received financial support from The Hørslev Foundation, King Christian the 10th Foundation and grant no. GM24211 from The National Institute of General Medical Sciences, National Institutes of Health.

K. B. Petersen · M. Rasmussen
Department of Pharmaceutics,
The Danish University of Pharmaceutical Sciences,
Copenhagen, Denmark

W. J. Jusko
Department of Pharmaceutical Sciences,
State University of New York at Buffalo,
Buffalo, NY, USA

K. Schmiegelow (✉)
Pediatric Clinic II,
The National University Hospital,
Rigshospitalet, 9 Blegdamsvej, DK-2100,
Copenhagen, Denmark
E-mail: kschmiegelow@rh.dk
Tel.: +45-35-451357
Fax: +45-35-454673

Introduction

Glucocorticoids have been used in the treatment of leukemias for more than 50 years [36]. The administration of glucocorticoids in acute lymphoblastic leukemia (ALL) induces apoptosis of leukemic cells [19], and a poor response to a 1-week prephase regimen of prednisone (and one intrathecal dose of methotrexate) has been shown to be of prognostic significance in childhood ALL [38]. Furthermore, the in vitro sensitivity to prednisolone, the active metabolite of prednisone, correlates with the residual leukemia following 4 weeks of remission induction therapy [46]. Despite the widespread use of glucocorticoids in childhood ALL, only a single pharmacokinetic study of prednisolone involving six patients has previously been reported, which showed an up to fourfold difference in

the pharmacokinetic parameters [6]. A study measuring the urinary excretion of prednisolone metabolites in 19 children with ALL showed a large interindividual variation [27].

Prednisolone exhibits dose-dependent pharmacokinetics with twofold changes in clearance and volume of distribution in the therapeutic dose range of 5–40 mg [44]. This dose-dependency is primarily due to nonlinear binding to the plasma protein transcortin and results in a change in protein binding from 95% at low prednisolone concentrations to about 60% at high concentrations. The pharmacokinetic parameters become essentially constant when they are based on the unbound concentrations [24, 42, 44]. Prednisolone undergoes reversible metabolism to the inactive prednisone. The reaction is catalyzed by 11β-hydroxysteroid dehydrogenase [8] and results in four to ten times higher concentrations of prednisolone in plasma [24, 44]. After an intravenous (i.v.) dose of prednisolone, 15–20% is excreted unchanged in the urine as the parent drug and 2–4% as prednisone. Urinary 6β-hydroxyprednisolone makes up 2–10% of an administered dose, and other metabolites include 20β-hydroxyprednisolone (5–7%), 20α-hydroxyprednisolone (3–5%) and 20β-hydroxyprednisone (<1%) [15, 16, 24, 44].

The population pharmacokinetic (PPK) approach offers the possibility of analyzing data from unbalanced and sparsely sampled studies [2, 30]. This is especially relevant for the pediatric population where intensive sampling is complicated by limited blood volumes [7].

The purpose of the present study was to evaluate the plasma protein binding and pharmacokinetics of prednisolone during therapeutic use in children with ALL using the population approach.

Materials and methods

Patients

The study included 23 children with ALL (aged 2 to 15 years) diagnosed at The University Hospital, Rigshospitalet, Copenhagen, Denmark, from February 2000 until September 2001. The patients did not differ significantly from the total number of patients diagnosed in this period ($n=35$) with respect to age, sex, immunophenotype, risk group assignment or white blood cell count (WBC) at diagnosis. The Regional Ethics Committee and the Danish Medicines Agency approved the study protocol. Verbal and written information about the study was given to the parents and written informed consent was obtained. If appropriate, informed consent was also obtained from the child.

Patient nos. 1–20 were treated according to the NOPHO ALL-1992 protocol (The Nordic Society for Pediatric Hematology and Oncology) and patient nos. 21–23 according to the NOPHO ALL-2000 protocol. Risk classification was based on WBC at diagnosis. Non-high-risk (n-HR) patients were those with WBC $<50\times10^9$/l, and high-risk (HR) patients were those with WBC $\geq50\times10^9$/l and/or the presence of CNS or testicular leukemia, T cell disease, a mediastinal mass, lymphomatous leukemia, chromosomal translocations t(9;22) or t(4;11), or a day-15 M3 or a day 29 M2 bone marrow [22].

Procedures

Prednisolone (60 mg/m^2 per day) was administered in three daily doses during the first 36 days of remission induction therapy. Concomitant chemotherapy consisted of weekly vincristine (2.0 mg/m^2 $\times$6), doxorubicin (40 mg/m^2 days 1 and 22; plus day 8 for HR patients on ALL-1992), and intrathecal methotrexate (10–12 mg days 1, 8, 15 and 29). Prednisolone was administered orally as either tablets or an oral solution (Prednisolone, SAD, Denmark) or i.v. as the hemisuccinate ester of prednisolone (Solu-Decortin H, Merck, Darmstadt, Germany). Intravenous prednisolone was administered as a bolus injection into a peripheral vein during a scheduled anesthetic procedure in order to avoid contamination of the central venous catheter used for blood sampling. Fasting was only required prior to i.v. administration. Between four and nine blood samples were obtained over an 8-h period following the morning dose of prednisolone on several days of induction therapy after both oral and i.v. administration. Blood samples were drawn into heparinized glass tubes, immediately cooled, and then centrifuged within 30 min (1500 *g*, 15 min at 5°C) for separation of plasma. Plasma samples were stored at −20°C until analysis.

Bioanalysis

Quantification of plasma prednisolone

Plasma prednisolone was determined based on the methods described by McBride et al. [29] and McWhinney et al. [31] with minor modifications. A Merck Hitachi high-performance liquid chromatography (HPLC) system with a Spherisorb RP-18 (250×4.6 mm, 5 μm) column and a LiChrospher 100 RP-18e (5 μm) guard column was used with UV detection at 254 nm. The mobile phase consisted of tetrahydrofuran/deionized filtered water (28:72 v/v). The flow rate was 1.0 ml/min and the temperature 31°C. Standard curves were linear in the range 0–2500 ng/ml. The intraday coefficient of variation (CV) was 7% at 20 ng/ml and 4% at 2500 ng/ml, whereas the interday CV ranged from 9% at 20 ng/ml to 5% at 2500 ng/ml. The detection limit (signal-to-noise ratio 3:1) was 6 ng/ml and recovery was >90%. After thawing, 1 ml plasma sample was mixed with 150 μl internal standard (fludrocortisone 150 ng) and 6 ml diethyl ether/chloroform (60:40 v/v). The tubes were tightly capped, vortexed for 15 s and centrifuged at 1800 *g* for 10 min. The organic phase was aspirated and evaporated to dryness under a stream of dry air. The residue was reconstituted in 150 μl mobile phase and 50 μl was injected onto the column.

Plasma protein binding of prednisolone

The plasma protein binding of prednisolone was determined as previously described by Chakraborty et al. using ultrafiltration and based on microplate scintillation counting (Topcount, Packard, Downers Grove, Ill.) [5].

Data analysis

Population pharmacokinetics

A two-compartment pharmacokinetic model with first-order absorption (for oral dosing) was used to describe the concentration versus time data for prednisolone. Due to the nonlinear binding of prednisolone to plasma proteins, the unbound fraction of prednisolone was determined for each sample, and only the unbound concentrations were modeled. Pharmacokinetic parameters estimated were k_a (first-order absorption rate constant), F (bioavailability), V_c (central volume of distribution), V_p (peripheral volume of distribution), CL (systemic clearance) and CL_d (intercompart-

mental clearance). Secondary parameters estimated were $t_{1/2}$ (half-life), AUC (area under the plasma concentration-time curve), V_{ss} (volume of distribution at steady state) and V_{β} (volume of distribution in the beta phase). The bioavailability was estimated by the simultaneous fitting of oral and i.v. data. Prednisolone doses administered within 48 h of blood sampling were entered in the model along with the time of dosing. For patients with more than one sampling day, data were entered as a continuous time stream. Constant CV error models were used for the interindividual and residual variabilities. The evaluation of the base model without covariates was based on goodness-of-fit criteria including visual inspection, examination of residuals, and the objective function value.

The effects of the following covariates on the PPK model parameters were examined: age, sex, risk group (n-HR versus HR), WBC at diagnosis, weight, height, body surface area (BSA) [20], body mass index, protocol day, fasting, the formulation of prednisolone (oral solution, tablet, i.v.), and the concentrations of albumin, serum creatinine, bilirubin, alanine aminotransferase (ALAT), and coagulation factors II + VII + X. The distribution and grouping of the covariates in the population are shown in Tables 1, 2, and 3. The weight/BSA ratio showed a twofold variation (22–40 kg/m^2) in the population indicating that BSA could not be predicted from weight alone. The effect of each covariate on the pharmacokinetic parameters was identified by forward univariate analysis. A decrease in the objective function value of at least 3.84 (χ^2, $P = 0.05$, df = 1) was considered statistically significant. Subsequently, all significant covariates identified in the univariate analysis were included in a multivariable model, and backward elimination was performed to exclude covariates that resulted in an increase in the objective function value of less than 6.63 (χ^2, $P = 0.01$, df = 1). The remaining, significant covariates were then included in the final PPK model.

Estimates of the patient-specific pharmacokinetic parameters were obtained from the final model by posthoc analysis. The PPK analysis was performed by nonlinear mixed-effects modeling using the first-order method in WinNonMix, version 2.0.1 (Pharsight Corporation, Mountain View, Calif.). Initial pharmacokinetic parameter estimates were obtained by naive pooled analysis. The validity of the AUC estimates was evaluated by comparison with estimates obtained by noncompartmental analysis using the trapezoidal method in WinNonlin, version 2.1 (Pharsight Corporation).

Table 1 Values of the continuous covariates in the population (*BSA* body surface area, *BMI* body mass index)

Covariate	Median[a]	Range
BSA (m^2)	0.8	0.6–1.8
BMI [kg/(height in m)^2]	16	13–27
Protocol day[b]	–	2–31
Albumin (g/l)[c]	36	23–46
Serum creatinine (mmol/l)[d]	0.042	0.027–0.076

[a]For patients with more than one sampling day, the average of the covariate was used to calculate the median for the population
[b]No median calculated for protocol day
[c]Twelve patients (52%) had decreased albumin according to age-related criteria
[d]Three patients (13%) had decreased creatinine according to age-related criteria

Table 2 The distribution and grouping of categorical covariates in the population

		No. (%)
Fasting	Yes	30 (64)
	No	17 (36)
Formulation	Oral solution	4 (8)
	Tablet	21 (45)
	Intravenous	22 (47)
Liver function[a]	1	8 (17)
	2	18 (38)
	3	21 (45)

[a]Based on ALAT, bilirubin and coagulation factors II + VII + X. *1* All normal; *2* At least one parameter abnormal, but ALAT and bilirubin increased less than three times and factors II + VII + X greater than 0.5 U/l; *3* ALAT or bilirubin increased more than three times and/or factors II + VII + X less than 0.5 U/l

Modeling of protein binding

The binding of prednisolone to the plasma proteins transcortin and albumin can be described by the following equation when no cortisol is present in plasma:

$$D_{total} = \frac{B_{max1} \cdot D_u}{1 + K_1 \cdot D_u} + K_{ns} \cdot D_u + D_u \quad (1)$$

where D_{total} and D_u represent the total and unbound concentrations of prednisolone in plasma, K_1 is the affinity constant for transcortin, and B_{max1} and K_{ns} are the binding capacities of transcortin and albumin. A population approach was used to obtain estimates of B_{max1}, K_1 and K_{ns}, and the albumin concentration was tested as a covariate for K_{ns}.

The modeling was performed independently of the PPK analysis. Protein binding measurements for each patient obtained on different days of induction therapy were pooled and modeled together, and the average albumin concentration was used. Constant CV error models were used for the interindividual and residual variabilities and the modeling was performed by the conditional first-order method using WinNonMix. Initial parameter estimates were obtained from the literature [4, 14, 33, 41].

Statistics

Apart from the covariate analysis, all statistical data analyses were performed using SAS, Version 8.02 (The SAS Institute, Cary, N.C.). The Wilcoxon rank sum test, the two-sample *t*-test and Spearman's rank correlation test were used to test possible differences between patient groups. *P*-values less than 0.05 were considered statistically significant.

Results

Population pharmacokinetics

Blood samples were obtained after 47 doses of prednisolone in 23 patients (25 oral and 22 i.v.; median dose 20.8 mg/m^2, range 18–24 mg/m^2). The patients were studied on 1 to 4 days during induction therapy, and 11 patients had samples taken after both oral and i.v. doses (Table 3). A total of 288 blood samples were obtained (median 12 per patient).

Plots of observed versus predicted unbound prednisolone concentrations are shown in Fig. 1 for the complete dataset, and examples of individual predicted versus observed plasma concentration-time profiles are shown in Fig. 2. Prednisolone appeared in plasma soon after both oral and i.v. administration, indicating that the oral

Table 3 Patient characteristics and estimated pharmacokinetic parameters of unbound prednisolone (*WBC* white blood cell count at diagnosis, *n-HR* non-high-risk group, *HR* high-risk group)

Patient	Age (years)	Sex (M/F)	Risk group (n-HR/HR)	WBC ($\times 10^9$/l)	Weight (kg)[a]	Height (cm)[a]	No. of samples/days	CL (l/h/m^2)	CL_d (l/h/m^2)	V_c (l/m^2)	V_p (l/m^2)	$t_{1/2}$ (h)	AUC (ng·h/ml)[b]
1	3.4	M	HR	25	14.7	103	4/1	32.3	19.1	39.3	41.9	2.8	611
2	8.5	M	HR	143	28.0	132	11/2	30.1	12.2	57.1	38.6	3.6	687
3	3.1	M	n-HR	25	17.0	101	6/1	31.8	17.9	44.3	46.7	3.3	693
4	5.2	M	HR	27	18.4	116	6/1	31.7	16.1	50.1	37.8	2.9	616
5	11.1	F	n-HR	9.4	42.0	152	16/3[c]	30.0	9.3	31.8	49.3	5.0	780
6	2.4	M	HR	48	14.2	98	6/1	28.0	19.9	36.4	37.9	2.7	1030
7	6.3	F	n-HR	11	18.5	108	6/1	33.7	16.5	27.0	48.9	3.3	632
8	5.2	F	n-HR	1.2	24.4	120	12/2	32.4	13.7	45.3	57.6	4.5	696
9	5.4	M	HR	65	18.2	113	18/3	31.8	16.4	78.4	43.0	3.6	644
10	9.2	M	HR	22	49.2	140	18/3[c]	33.7	8.9	55.8	41.1	4.3	646
11	7.4	F	n-HR	2.6	28.3	133	17/3[c]	28.4	12.1	10.5	43.9	3.7	819
12	7.4	M	n-HR	1.4	26.0	133	21/3[c]	32.1	12.6	37.8	48.5	4.0	759
13	13.7	M	n-HR	6.0	44.1	165	12/2[c]	29.9	8.8	53.7	53.8	5.8	784
14	4.9	M	n-HR	13	21.1	110	11/2	26.6	15.5	52.6	87.2	6.8	1004
15	9.8	F	HR	628	36.0	131	11/2[c]	26.3	10.8	37.9	50.1	4.9	1012
16	2.7	M	n-HR	6.0	13.7	92	10/2	24.3	20.9	45.2	47.3	3.6	855
17	6.8	M	HR	206	19.3	113	21/3[c]	24.8	15.9	23.0	40.2	3.2	811
18	4.3	M	HR	790	17.9	112	12/2[c]	36.0	16.6	45.5	39.0	2.7	687
19	15.2	F	n-HR	16	73.7	166	32/4[c]	32.3	6.7	63.0	65.3	7.2	695
20	5.7	F	n-HR	2.7	19.7	122	12/2[c]	33.1	15.1	28.8	37.8	2.7	803
21	2.9	M	n-HR	5.5	13.1	99	5/1	33.8	20.6	45.0	39.3	2.6	659
22	3.8	M	n-HR	0.7	15.0	102	14/2[c]	32.0	19.0	43.7	33.1	2.5	664
23	5.3	M	HR	117	24.2	125	7/1	30.8	13.5	38.3	36.0	3.0	743
Median	5.4			16	19.7	116	12/2	31.8	15.5	44.3	43.0	3.6	696
Range	2.4–15.2	16/7	13/10	0.7–790	13.1–73.7	92–166	4–32/1–4	24.3–36.0	6.7–20.9	10.5–78.4	33.1–87.2	2.5–7.2	611–1030

[a]For patients with more than one sampling day, the average value is shown
[b]Normalized based on a dose of 20 mg/m^2
[c]Samples obtained after both oral and i.v. administration

absorption and the hydrolysis of the i.v. ester occur rapidly. The i.v. doses yielded a biexponential disposition, and for the oral doses the maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) showed median values of 221 ng/ml and 1.3 h. Further, the plasma profiles for oral and i.v. dosing indicated a high oral bioavailability. Inclusion of an oral absorption lag time in the PPK model was attempted, but resulted in negligible estimates.

The estimated pharmacokinetic parameters are shown in Table 3 and their interindividual variabilities in Table 4. The interindividual variability in k_a and CL_d could not be accurately estimated and only the population estimates were obtained. Complete absorption of prednisolone after oral administration was seen with an estimated F of 1.0 (range 0.9–1.2) and a negligible interindividual variability of 0.1–0.2%. The absorption rate constant, k_a, was estimated to 1.35 h^{-1}. The estimated CL (median 32 l/h per m^2) and dose-normalized AUC (median 696 ng·h/ml) differed less than twofold among individuals, whereas V_c, V_p, V_{ss} and V_β showed a three- to sevenfold variation with median values of 44, 43, 86 and 150 l/m^2 and ranges of 54–140 and 110–393 l/m^2 for V_{ss} and V_β. Further, a median $t_{1/2}$ of 3.6 h with an almost threefold range from 2.5 to 7.2 h was observed. For 21 out of the 23 patients, the AUC estimates obtained by nonlinear mixed-effects modeling were within ±20% of the estimates obtained by noncompartmental analysis, whereas they were within ±50% for the remaining patients (data not shown).

Tables 5 and 6 illustrate the influence of patient covariates on the pharmacokinetic parameters. The V_c and V_p values were linearly correlated with weight, while CL was proportional to BSA. Taking these covariates into account decreased the interindividual variability for the final model compared to the base model as shown in Table 4. This effect was especially pronounced for CL where the variability decreased from 28% to 14% after inclusion of BSA. In the final model, the patient-specific CL values normalized for BSA were not related to sex (medians: male 31.7 l/h per m^2, female 32.3 l/h per m^2; $P = 0.84$), age at diagnosis ($r_s = -0.05$; $P = 0.81$; Fig. 3), WBC at diagnosis ($r_s = -0.27$; $P = 0.28$), risk group (medians: n-HR 31.8 l/h per m^2, HR 31.7 l/h per m^2; $P = 0.48$) or body weight ($r_s = -0.07$; $P = 0.74$). The residual variability decreased from 25% in the base model to 21% and 20% for oral and i.v. dosing, respectively, in the final model.

Modeling of protein binding

The plasma protein binding of prednisolone was nonlinear in the observed concentration range as shown in Fig. 4. The percent of protein-bound prednisolone in plasma ranged from 93% at low concentrations to 35–66% at concentrations above 1000 ng/ml. At low concentrations prednisolone primarily binds to transcortin with high affinity and low capacity. Binding to albumin

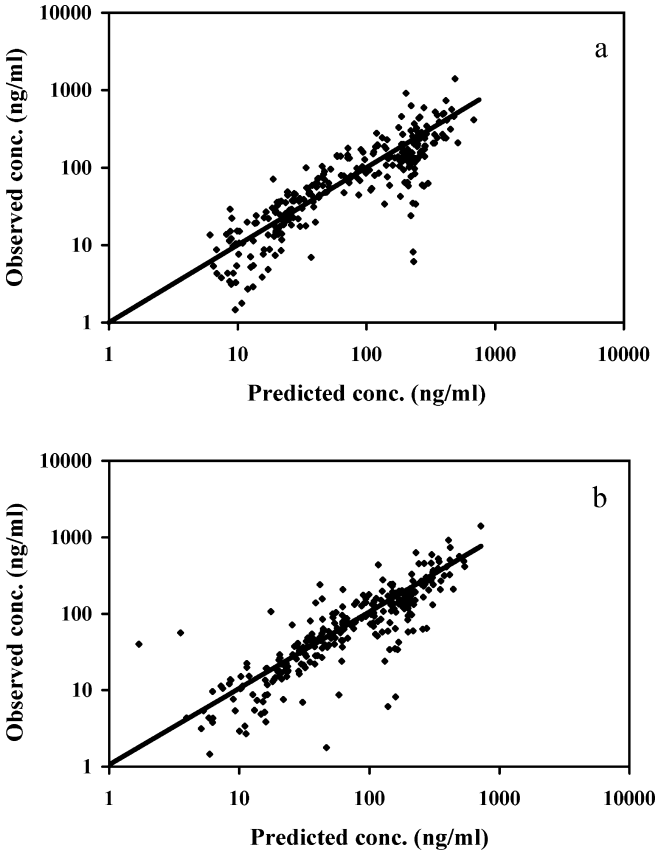

Fig. 1a, b Scatterplots showing the relationship between the observed unbound prednisolone concentrations and (**a**) the predicted population concentrations or (**b**) the predicted individual concentrations for the final covariate model

Table 4 Interindividual variability (%CV) in pharmacokinetic parameters

	CL (l/h/m^2)	V_c (l/m^2)	V_p (l/m^2)
Base model without covariates	28%	60%	53%
Final covariate model	14%	50%	42%

with low affinity and high capacity predominates when transcortin becomes saturated at higher concentrations. The population estimates of the protein binding parameters along with their interindividual variabilities are shown in Table 7. Apart from B_{max1} with an interindividual variability of 27%, the variabilities were negligible. Further, albumin was not found to be a covariate for K_{ns} ($P = 0.71$). The residual variability was estimated to 9%.

Discussion

In the present study, a PPK model was applied to assess the determinants of the pharmacokinetics of prednisolone in children with ALL. The simultaneous fitting of oral and i.v. data made it possible to estimate the bioavailability based on all measurements and not only on the 11 patients given both formulations. Even though the route of administration and the fasting status of the patients were not randomized, the complete bioavailability seen in the present study indicates that the systemic exposure to prednisolone is independent of these factors in children with ALL. Studies of

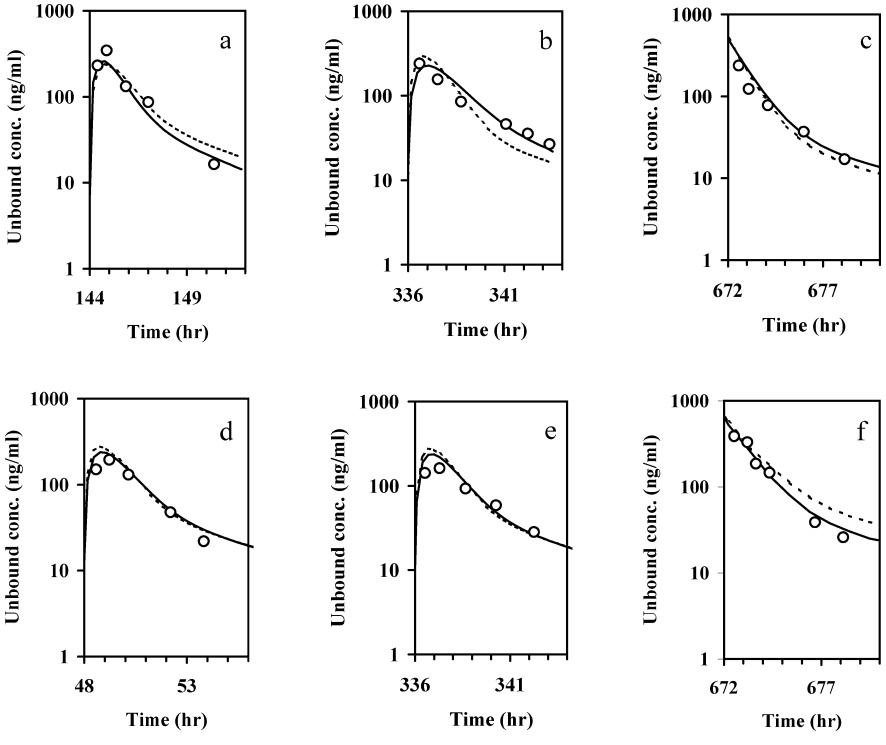

Fig. 2a–f Predicted versus observed plasma concentration-time profiles for (**a**) patient 5 (oral dose at 144 h, day 7), (**b**) patient 13 (oral dose at 336 h, day 15), (**c**) patient 13 (i.v. dose at 672 h, day 29), (**d**) patient 8 (oral dose at 48 h, day 3), (**e**) patient 8 (oral dose at 336 h, day 15), and (**f**) patient 14 (i.v. dose at 672 h, day 29). (○ observed measurement, — predicted profile for the individual patient, --- predicted population profile)

Table 5 Regression models describing relationships between unbound prednisolone pharmacokinetic parameters and patient covariates in the forward univariate analysis (*BSA* body surface area in m^2, *WT* weight in kg, ΔOBJF change in the objective function value upon inclusion of the covariate)

	Run no.			
	1	2	3	4
Regression model[a]	$CL = \theta_1 \times BSA$	$V_c = \theta_2 + \theta_3 \times WT$	$V_p = \theta_4 + \theta_5 \times WT$	$CL = (\theta_1 \times BSA) + \theta_6 \times sex$
Estimated regression coefficients				
θ_1	22.6	30.4	30.7	29.7
θ_2		12.8	14.0	12.4
θ_3		0.82	0.73	0.81
θ_4			17.3	18.0
θ_5			1.35	1.16
θ_6				5.2
ΔOBJF	−90	−26	−16	−6.1
P-value	< 0.001	< 0.001	< 0.001	< 0.05

[a]The most significant regression model for the particular run

Table 6 Regression models describing relationships between unbound prednisolone pharmacokinetic parameters and patient covariates in the final covariate model (*BSA* body surface area in m^2, *WT* weight in kg)

Regression model	Estimated regression coefficients	
		Coefficients of variation
$CL\ (l/h) = \theta_1 \times BSA$		
θ_1	30.7	6.5
$V_c\ (l) = \theta_2 + \theta_3 \times WT$		
θ_2	14.0	34
θ_3	0.73	21
$V_p\ (l) = \theta_4 + \theta_5 \times WT$		
θ_4	17.3	54
θ_5	1.35	35

Fig. 3 Relationship between age and prednisolone clearance for male (◆) and female (◇) patients

prednisolone in patients and volunteers have shown an oral bioavailability of 60–100% [17, 40, 44] and complete absorption has been reported in children with inflammatory bowel disease [33, 34]. Malabsorption in childhood ALL has previously been described by some [9, 35] but not all authors [23], but even if malabsorption was present during induction therapy, this apparently did not influence the bioavailability of prednisolone, and oral administration seems appropriate in this population.

Upon i.v. administration, the prednisolone hemisuccinate ester is rapidly hydrolyzed with an essentially complete conversion to prednisolone and a half-life of about 18 min [10]. In the present study, no stabilizing agent was added to the blood samples, but as the samples were cooled immediately and centrifuged within 30 min only minimal additional hydrolysis could have taken place. Besides, the peak concentration of prednisolone appeared as early as 6–10 min after i.v. administration in our study indicating rapid hydrolysis.

Pharmacokinetic studies of prednisolone in children have been carried out over the past 25 years [1, 6, 11, 13, 18, 21, 32, 33, 34, 40, 43, 45], but the present study is the first population approach. Further, as shown in Table 8, the pharmacokinetics of unbound prednisolone have been described in only a few pediatric studies. The median CL seen in the present study (Table 3) was only half the value observed by Choonara et al. in six children with ALL [6]. Our value of CL was, however, in accordance with that seen in asthmatic children [43] and in children with inflammatory bowel disease [33], whereas children with nephrotic syndrome show both higher [18] and lower [32] clearance values (Table 8). Further, the estimates of both CL and V_c in the present study were generally in agreement with those observed in adults [5, 28, 44, 47]. Regarding estimates of volume of distribution in the pediatric population, our results were lower than those previously reported in ALL [6], but higher than seen in nephrotic children [32] (Table 8).

The $t_{1/2}$ of 3.6 h in the present study was longer than mean values previously seen in the pediatric population (1.3–2.9 h) [6, 18], but ranges of 1–6 h have been reported [13, 21]. The longest $t_{1/2}$ of 7.2 h in our study was seen in the oldest patient (15 years). Apart from the study in ALL patients (median age 4.8 years) [6], the children studied in the studies listed in Table 8 were

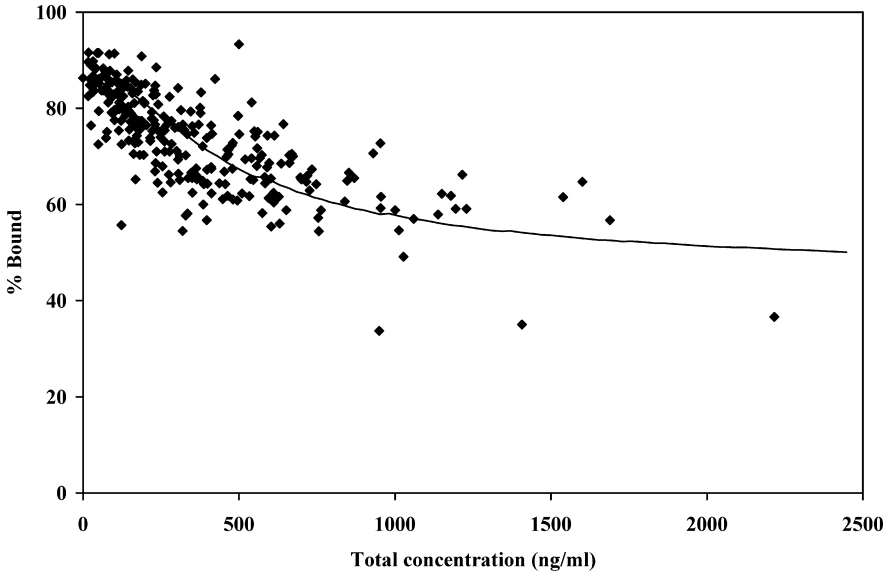

Fig. 4 Relationship between the total concentration of prednisolone and percentage prednisolone bound to plasma proteins. The line represents the predicted population profile

older (median ages ranging from 10 to 18 years) than our population. Since drug clearance is often relatively higher in younger children compared to adolescents and adults [3, 12], our population seems to have had a lower clearance than would be expected based on the age distribution. Relling et al. hypothesized that hepatic leukemic infiltration at the time of diagnosis could generally decrease drug clearances [39]. However, tumor burden (WBC at diagnosis) was not found to affect the pharmacokinetic parameters in the present study. Likewise, no patients received concurrent medication known to alter prednisolone metabolism, and prednisolone itself does not induce its own metabolism in humans [24, 37]. Further, no covariate linked to liver function arose from the present study even though most patients had increased values of ALAT due to tumor lysis.

Table 7 Prednisolone plasma protein binding parameters and interindividual variability expressed as coefficients of variation

Parameter	Estimate		Interindividual variability (%)
		Coefficient of variation	
B_{max1}	6.77	5.6	27
K_1 (M^{-1})	0.95×10^7	18	1.3
K_{ns}	0.80	24	0.6

The covariate testing showed that BSA had a statistically significant effect on CL while body weight had effect on both volumes of distribution (Tables 5 and 6). Upon inclusion of BSA as a covariate for CL, the influence of other correlated covariates (e.g. age, weight) disappeared. Likewise, adding weight as a covariate for V_c and V_p made the influence of age and BSA disappear. During the backward elimination analysis, sex was excluded as a covariate for CL. Even though the criterion for significance during backward elimination ($P = 0.01$) was strict in the present study, the minor influence of sex was probably due to the higher median age of the girls included in the present study (Fig. 3). The interindividual variability decreased in the final model after addition of explanatory covariates (Table 4). The model seemed to capture the interindividual variability for CL very well when BSA was included, but for V_c and V_p the interindividual variability remained high (50% and 42%) after weight had been taken into account.

The residual variability expresses both the intraindividual variability and the variability due to model misspecification, sampling and assay errors, and patient compliance. By obtaining blood samples on several occasions for most of the patients, a substantial intraindividual variability would thus influence the estimated residual variability. This is essential as a large intraindividual variability can limit the potential feasibility of

Table 8 Pharmacokinetic studies of unbound prednisolone in pediatric patients

Reference	Patients	Dose	CL (l/h/m^2)	V (l/m^2)	$t_{1/2}$ (h)
6	6 ALL (3–12 years)	10–20 mg oral	63[a] (37–111)	116[a] (54–206) (V_c)	1.3
18	9 nephrotic syndrome (4–15 years)	Approx. 2 mg/kg oral	57[a,b]	–	2.9
32	11 nephrotic syndrome (4–16 years)	30–65 mg i.v.	6.7 (4–15)	51 (29–74) (V_β)	2.1
43	10 asthmatics (8–12 years)	40 mg i.v.	39[a]	–	2.5
33	7 inflammatory bowel disease (8–20 years)	20–40 mg oral, i.v.	27[a]	–	2.5

[a]Calculated from pharmacokinetic parameters or demographic data in the article
[b]Result in l/h/m^2 obtained by multiplying the CL value in l/h/kg by 30

therapeutic drug monitoring [25]. In the present study, both the administration of prednisolone and blood sampling were performed or supervised by study personnel in order to minimize variations related to these factors. The residual variability of about 20% in our study indicates that the pharmacokinetics of prednisolone remained stable throughout induction therapy despite the intensive chemotherapy regimen and the disease process itself. This is in accordance with a study by Langhoff et al. in which 11 patients with renal transplants or collagenosis were studied on two occasions separated by 45–325 days and showed only minor changes in pharmacokinetic parameters [26].

Based on the low variation in the estimated AUC values, an equivalent systemic exposure to prednisolone was seen in the patients after a dose of 20 mg/m^2 administered three times daily. This is in accordance with previous findings in the pediatric population [18, 33]. Further, simulations performed with dosage regimens of 2–2.5 mg/kg resulted in larger, but statistically insignificant, variations in the estimated AUC values. Thus, a dosage regimen based on BSA seems appropriate for the administration of prednisolone in childhood ALL to obtain a similar systemic exposure in all patients. However, whether the exposure and dosage regimen used in the present protocol would result in an optimal antileukemic efficacy is unknown.

The protein binding of prednisolone was found to be nonlinear in the observed concentration range, as previously reported [24, 42, 44]. The competitive binding of cortisol to transcortin and albumin could be excluded from the analysis as the dosing regimen of prednisolone produced pronounced suppression, and we observed essentially negligible concentrations of endogenous cortisol.

In general, the estimated protein binding parameters (Table 7) were comparable to but slightly lower than previously published data, where mean values ranging from 8–30 (B_{max1}), 1.5–5.1×10^7 (K_1) and 0.9–1.45 (K_{ns}) have been reported [4, 14, 33, 41]. Albumin was not found to be a covariate for K_{ns}. A reason for this might have been that the twofold difference in albumin concentrations in the present study was too small to cause significant changes in protein binding. Further, all samples from an individual patient were pooled without taking the difference in albumin concentrations on different sampling days into account. However, re-analyzing the data as 47 individual sampling days only caused minor (3–5%) changes in the parameter estimates and did not make albumin a significant covariate for K_{ns} ($P = 0.94$).

In conclusion, the nonlinear plasma protein binding and the pharmacokinetics of prednisolone in children with ALL were evaluated using the population approach. The study showed complete oral bioavailability, a lower clearance, and a longer half-life than previously reported. The pharmacokinetic parameters remained stable throughout induction therapy and the patients experienced similar systemic exposures to prednisolone. Thus, individualized dosing of prednisolone based on AUC values does not seem indicated in childhood ALL, even though further validation of the predictive performance of the PPK model is needed.

Acknowledgements The authors thank Ms. Suzette Mis for performing the protein binding assays.

References

1. Bartoszek M, Brenner AM, Szefler SJ (1987) Prednisolone and methylprednisolone kinetics in children receiving anticonvulsant therapy. Clin Pharmacol Ther 42:424
2. Beal SL, Sheiner LB (1982) Estimating population kinetics. Crit Rev Biomed Eng 8:195
3. Blanco JG, Harrison PL, Evans WE, Relling MV (2000) Human cytochrome P450 maximal activities in pediatric versus adult liver. Drug Metab Dispos 28:379
4. Boudinot FD, Jusko WJ (1984) Plasma protein binding interaction of prednisone and prednisolone. J Steroid Biochem 21:337
5. Chakraborty A, Blum RA, Mis SM, Cutler DL, Jusko WJ (1999) Pharmacokinetic and adrenal interactions of IL-10 and prednisolone in healthy volunteers. J Clin Pharmacol 39:624
6. Choonara I, Wheeldon J, Rayner P, Blackburn M, Lewis I (1989) Pharmacokinetics of prednisolone in children with acute lymphoblastic leukemia. Cancer Chemother Pharmacol 23:392
7. Collart L, Blaschke TF, Boucher F, Prober CG (1992) Potential of population pharmacokinetics to reduce the frequency of blood sampling required for estimating kinetic parameters in neonates. Dev Pharmacol Ther 18:71
8. Conti M, Frey FJ, Escher G, Marone C, Frey BM (1994) Renal handling of prednisolone/prednisone: effect of steroid dose and 11β-hydroxysteroid dehydrogenase. Nephrol Dial Transplant 9:1622
9. Craft AW, Kay HEM, Lawson DN, McElwain TJ (1977) Methotrexate-induced malabsorption in children with acute lymphoblastic leukemia. BMJ 2:1511
10. Derendorf H, Rohdewald P, Möllmann H, Rehder J, Barth J, Neveling D (1985) Pharmacokinetics of prednisolone after high doses of prednisolone hemisuccinate. Biopharm Drug Dispos 6:423
11. Dove AM, Szefler SJ, Hill MR, Jusko WJ, Larsen GL, Accurso FJ (1992) Altered prednisolone pharmacokinetics in patients with cystic fibrosis. J Pediatr 120:789
12. Evans WE, Relling MV, de Graaf S, Rodman JH, Pieper JA, Christensen ML, Crom WR (1989) Hepatic drug clearance in children: studies with indocyanine green as a model substrate. J Pharm Sci 78:452
13. Faure C, André J, Pelatan C, Munck A, Giraud M, Cèzard JP, Jacqz-Aigrain E (1998) Pharmacokinetics of intravenous methylprednisolone and oral prednisone in paediatric patients with inflammatory bowel disease during the acute phase and in remission. Eur J Clin Pharmacol 54:555
14. Ferry JJ, Wagner JG (1987) The nonlinear pharmacokinetics of prednisone and prednisolone. II. Plasma protein binding of prednisone and prednisolone in rabbit and human plasma. Biopharm Drug Dispos 8:261
15. Frey FJ, Frey BM (1983) Urinary 6β-hydroxyprednisolone excretion indicates enhanced prednisolone catabolism. J Lab Clin Med 101:593
16. Garg V, Jusko WJ (1991) Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-performance liquid chromatography. J Chromatogr 567:39
17. Garg V, Jusko WJ (1994) Bioavailability and reversible metabolism of prednisone and prednisolone in man. Biopharm Drug Dispos 15:163

18. Gatti G, Perucca E, Frigo GM (1984) Pharmacokinetics of prednisone and its metabolite prednisolone in children with nephrotic syndrome during the active phase and in remission. Br J Clin Pharmacol 17:423
19. Gaynon PS, Lustig RH (1995) The use of glucocorticoids in acute lymphoblastic leukemia of childhood. J Pediatr Hematol Oncol 17:1
20. Gehan EA, George SL (1970) Estimation of human body surface area from height and weight. Cancer Chemother Rep 54:225
21. Green OC, Winter RJ, Kawahara FS, Phillips LS, Lewy PR, Hart RL, Pachman LM (1978) Pharmacokinetic studies of prednisolone in children. J Pediatr 93:299
22. Gustafsson G, Schmiegelow K, Forestier E, Clausen N, Glomstein A, Jonmundsson G, Mellander L, Mäkipernaa A, Nygaard R, Saarinen-Pikkhala UM (2000) Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of CNS irradiation. Leukemia 14:2267
23. Halton J, Atkinson SA, Bradley CB, Dawson S, Barr RD (1993) Acute lymphoid leukemia. No evidence of consistent chemotherapy-induced intestinal malabsorption. J Pediatr Henatol Oncol 15:271
24. Jusko WJ, Ludwig EA (1992) Corticosteroids. In: Evans WE, Schentag JJ, Jusko WJ (eds) Applied pharmacokinetics, 3rd edn. Applied Therapeutics, Vancouver, p 27-I
25. Karlsson MO, Sheiner LB (1993) The importance of modeling interoccasion variability in population pharmacokinetic analyses. J Pharmacokinet Biopharm 21:735
26. Langhoff E, Flachs H, Ladefoged J, Hvidberg EF (1984) Intraindividual consistency of prednisolone kinetics during long-term prednisone treatment. Eur J Clin Pharmacol 26:651
27. Lilleyman JS, French AJ, Young IP (1981) Variation in 17 oxogenic steroid excretion following oral prednisolone in children with lymphoblastic leukemia. Oncology 38:274
28. Magee MH, Blum RA, Lates CD, Jusko WJ (2001) Prednisolone pharmacokinetics and pharmacodynamics in relation to sex and race. J Clin Pharmacol 41:1180
29. McBride JH, Rodgerson DO, Park SS, Reyes AF (1991) Rapid liquid-chromatographic method for simultaneous determination of plasma prednisone, prednisolone, and cortisol in pediatric renal-transplant recipients. Clin Chem 37:643
30. McLachlan AJ (1996) Sparse drug concentration data analysis using a population approach: a valuable tool in clinical pharmacology. Clin Exp Pharmacol Physiol 23:995
31. McWhinney BC, Ward G, Hickman PE (1996) Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine. Clin Chem 42:979
32. Miller PFW, Bowmer CJ, Wheeldon J, Brocklebank JT (1989) Pharmacokinetics of prednisolone in children with nephrosis. Arch Dis Child 65:196
33. Milsap RL, George DE, Szefler SJ, Murray KA, Lebenthal E, Jusko WJ (1983) Effect of inflammatory bowel disease on absorption and disposition of prednisolone. Dig Dis Sci 28:161
34. Olivesi A (1985) Normal absorption of oral prednisolone in children with active inflammatory bowel disease, including cases with proximal to distal small bowel involvement. Gastroenterol Clin Biol 9:564
35. Pearson AD, Craft AW, Pledger JV, Eastham EJ, Laker MF, Pearson GL (1984) Small bowel function in acute lymphoblastic leukemia. Arch Dis Child 59:460
36. Pearson OH, Eliel LP (1950) Use of pituitary adrenocorticotropic hormone (ACTH) and cortisone in lymphomas and leukemias. JAMA 144:1349
37. Pichard L, Fabre I, Daujat M, Domergue J, Joyeux H, Maurel P (1992) Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin A oxidase activity in primary cultures of human hepatocytes. Mol Pharmacol 41:1047
38. Reiter A, Schrappe M, Ludwig WD, Hiddeman W, Sauter S, Henze G, Zimmermann M, Lampert F, Havers W, Niethammer D (1994) Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 84:3122
39. Relling MV, Crom WR, Pieper JA, Cupit GC, Rivera GK, Evans WE (1987) Hepatic drug clearance in children with leukemia: changes in clearance of model substrates during remission-induction therapy. Clin Pharmacol Ther 41:651
40. Rocci ML, Assael BM, Appiani AC, Edefonti A, Jusko WJ (1982) Effect on nephrotic syndrome on absorption and disposition of prednisolone in children. Int J Pediatr Nephrol 3:161
41. Rocci ML, D'Ambrosio R, Johnson NF, Jusko WJ (1982) Prednisolone binding to albumin and transcortin in the presence of cortisol. Biochem Pharmacol 31:289
42. Rohatagi S, Barth J, Möllmann H, Hochhaus G, Soldner A, Möllmann C, Derendorf H (1997) Pharmacokinetics of methylprednisolone and prednisolone after single and multiple oral administration. J Clin Pharmacol 37:916
43. Rose JQ, Nickelsen JA, Ellis EF, Middleton W, Jusko WJ (1981) Prednisolone disposition in steroid-dependent asthmatic children. J Allergy Clin Immunol 67:188
44. Rose JQ, Yurchak AM, Jusko WJ (1981) Dose dependent pharmacokinetics of prednisone and prednisolone in man. J Pharmacokinet Biopharm 9:389
45. Rostin M, Barthe P, Houin G, Alvinerie M, Bouissou F (1990) Pharmacokinetics of prednisolone in children with the nephrotic syndrome. Pediatr Nephrol 4:470
46. Schmiegelow K, Nyvold C, Seyfarth J, Pieters R, Rottier MMA, Knabe N, Ryder LP, Madsen HO, Svejgaard A, Kaspers GJL (2001) Post-induction residual leukemia in childhood acute lymphoblastic leukemia quantified by PCR correlates with in vitro prednisolone resistance. Leukemia 15:1066
47. Wald JA, Law RM, Ludwig EA, Sloan RR, Middleton E, Jusko WJ (1992) Evaluation of dose-related pharmacokinetics and pharmacodynamics of prednisolone in man. J Pharmacokinet Biopharm 20:567