DOI:10.1111/j.1365-2125.2007.03003.x

Developmental pharmacokinetics of ciclosporin – a population pharmacokinetic study in paediatric renal transplant candidates

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What is already known about this subject

- Ciclosporin is an immunosuppressant drug with a narrow therapeutic index and large variability in pharmacokinetics.
- It is likely that the inter- and intraindividual variability in ciclosporin pharmacokinetics and dose requirements is even larger in children than in adults as a result of variation in biological maturation status.
- However, data on the developmental pharmacokinetics of ciclosporin, as well as other CYP3A4 substrate drugs, in children are scarce.

What this study adds

- Adult CYP3A4 activity seems to be reached by the age of 6–12 months, and allometrically scaled body weight is a good predictor of the hepatic clearance of ciclosporin, a CYP3A4 substrate.
- Ciclosporin oral bioavailability, known previously to display large interindividual variability, is not influenced by age.
- These conclusions were reached using a robust modelling approach (NONMEM) with rich paediatric pharmacokinetic data collected after full i.v. and p.o. profiles.

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Keywords
ciclosporin (cyclosporine A), CYP3A4, developmental, paediatric, population pharmacokinetics, renal transplantation

Received
19 December 2006
Accepted
15 May 2007
Published *OnlineEarly*
27 July 2007

Aims
To use population pharmacokinetic modelling to characterize the influence of developmental and demographic factors on the pharmacokinetic variability of ciclosporin.

Methods
Pharmacokinetic modelling was performed in NONMEM using a dataset comprising 162 pretransplant children, aged 0.36–17.5 years. Ciclosporin was given intravenously (3 mg kg^{-1}) and orally (10 mg kg^{-1}) on separate occasions followed by blood sampling for 24 h.

Results
A three-compartment model with first-order absorption without lag-time best described the pharmacokinetics of ciclosporin. The most important covariate affecting systemic clearance (CL) and distribution volume (*V*) was body weight (BW; scaled allometrically), responsible for a fourfold difference in uncorrected ciclosporin CL and a sixfold difference in ciclosporin *V*. The other significant covariates, haematocrit, plasma cholesterol and creatinine, were estimated to explain 20–30% of interindividual differences in CL and *V* of ciclosporin. No age-related changes in oral bioavailability or in BW-normalized *V* were seen. The BW-normalized CL (CL/BW) declined with age and prepubertal children (<8 years) had an approximately 25% higher CL/BW than did older children. Normalization of CL for allometric BW ($BW^{3/4}$) removed its relationship to age.

Conclusion
The relationship between CL and allometric BW is consistent with a gradual reduction in relative liver size, until adult values, and a relatively constant CYP3A4 content in the liver from about 6–12 months of age to adulthood. Ciclosporin oral bioavailability, known previously to display large interindividual variability, is not influenced by age. These findings can enable better individualization of ciclosporin dosing in infants, children and adolescents.

Introduction

Ciclosporin is a drug with a narrow therapeutic index and large interindividual variability in pharmacokinetics [1, 2]. Ciclosporin is highly bound to blood cells and plasma lipoproteins and has a large volume of distribution. Its unbound fraction in plasma in adult kidney transplant patients is about 3–12% [3]. Ciclosporin is extensively metabolized to about 30 metabolites by the cytochrome P450 3A (CYP3A) enzymes and it has a low oral bioavailability, largely explained by intestinal and hepatic presystemic metabolism [4–6]. The major elimination pathway for ciclosporin metabolites is the biliary route, whereas only 6% of the metabolites are excreted renally [7, 8].

A few, relatively small ($n = 5$–50), conventional studies of paediatric renal transplant recipients describing the pharmacokinetics of ciclosporin have been published [9, 10]. They have considerable methodological differences and the pharmacokinetic values obtained show variability within and between studies. In particular, published data concerning young children (≤5 years) are limited, and virtually no data exist concerning patients <1 year old. Therefore, knowledge of the developmental and other factors influencing the pharmacokinetics of ciclosporin has been limited.

Subtherapeutic ciclosporin concentrations are associated with an increased incidence of acute rejection or graft loss, whereas supratherapeutic concentrations may lead to adverse effects, mainly nephrotoxicity [11]. Therefore, ciclosporin treatment in children is usually initiated with body weight (BW)-based dosing and the individual dose established on the basis of monitoring of ciclosporin exposure [12–17]. However, inability to establish the correct individual dose often results in a high degree of variability of drug exposure over time, which may lead to the occurrence of chronic nephropathy [15].

The possibility of determining the patient characteristics that affect the pharmacokinetics of ciclosporin before transplantation and of employing Bayesian forecasting could help clinicians make better predictions of individual drug doses in the immediate post-transplant period [18]. It is likely that the inter- and intraindividual variability in ciclosporin pharmacokinetics and dose requirements is even larger in children than in adults as a result of variation in biological maturation [9]. However, data about the developmental pharmacokinetics of ciclosporin, and CYP3A4 substrate drugs in general, are scarce [19, 20].

The aim of this study was to characterize the magnitude of the effects of developmental and other factors on ciclosporin pharmacokinetics in a paediatric population using population pharmacokinetic modelling, in order to improve the possibilities of early individualization of ciclosporin dosing.

Patients and methods

Patients and ciclosporin concentration measurements

At the Hospital for Children and Adolescents of the University of Helsinki, the individual starting doses of ciclosporin in paediatric renal transplant patients have been determined using predictions obtained by detailed pretransplantation pharmacokinetic studies since 1988 [21].

Pretransplantation pharmacokinetic data were collected from 166 children who had been studied on average 10 months prior to transplantation (the average waiting time for a renal transplant in this clinic) during 1988–2005. Neither the standard medication for renal insufficiency nor any other concomitant medications were interrupted for the study. The study protocol was approved by the Ethics Committee of our hospital.

In the pharmacokinetic study, patients entered the clinic in the morning after an overnight fast. Ciclosporin was given as an intravenous (i.v.) 4-h infusion (3 mg kg^{-1}, Sandimmun; Novartis, Basel, Switzerland) and a single oral dose [10 mg kg^{-1}, Sandimmun (conventional oral formulation) or Sandimmun Neoral (microemulsion formulation); Novartis] with an interval of at least 24 h between the doses (usually 48–72 h). One patient was accidentally given only 1 mg kg^{-1} of ciclosporin orally. The oral pharmacokinetic study was conducted in 155 patients after the i.v. study and in 11 patients before the i.v. study. Two patients underwent two i.v. studies and one patient underwent only the oral study. Ciclosporin concentrations were determined from 1-ml ethylenediamine tetraaceticacid blood samples drawn at 0, 1, 2, 3, 4, 6, 9, 12, 16 and 24 h after the oral dose, and before (0 h), in the middle of (2 h) and at the end of the i.v. infusion (4 h) and 1, 2, 3, 4, 6, 9, 12, 16 and 24 h after the end of infusion. The exact time of each blood sample was written down. Blood sampling was carried out with an indwelling cannula, held open with an obturator or slow glucose infusion. The blood samples were stored at 4°C and analysed within 3 days.

The whole blood ciclosporin concentrations were determined using a specific monoclonal radioimmunoassay (RIA): Sandimmune Kit®, Sandoz, Basel, Switzerland until May 1994; Cyclo-Trac Kit®, Incstar, Stillwater MN, USA until December 1999 and Cyclo-Trac SP-Whole Blood®, DiaSorin, Saluggia, Italy thereafter (the Cyclo-Trac kit by Incstar and by DiaSorin are technically the same kit). The limit of detection of the assays was approximately 5 μg l^{-1}. The within-run and

Table 1
Demographics and biochemical characteristics of the 162 paediatric renal transplant candidates included in model building

Variable	*N*	Mean ± SD	Range
Body weight (kg)	162	22.2 ± 15.7	6.9–64
Height (cm)	145	107.3 ± 35.5	61.0–176
Age (years)	162	6.3 ± 5.7	0.36–17.5
Blood haemoglobin ($g\,l^{-1}$)	148	104 ± 23	47–154
Haematocrit (%)	148	31 ± 7	15–48
Serum protein content ($g\,l^{-1}$)	126	64 ± 8	42–83
Serum prealbumin ($mg\,l^{-1}$)	119	373 ± 92	179–668
Serum creatinine ($\mu mol\,l^{-1}$)	145	592 ± 252	165–1669
Serum aspartate aminotransferase ($U\,l^{-1}$)	128	35 ± 23	10–247
Serum alanine aminotransferase ($U\,l^{-1}$)	129	34 ± 54	5–564
Serum alkaline phosphatase ($U\,l^{-1}$)	132	682 ± 407	104–1952
Total plasma triglycerides, fasting value ($mmol\,l^{-1}$)	133	2.9 ± 1.5	0.68–8.2
Total plasma cholesterol, fasting value ($mmol\,l^{-1}$)	132	5.7 ± 1.6	3.1–9.8
Low-density lipoprotein, fasting value ($mmol\,l^{-1}$)	112	3.3 ± 1.3	1.2–7.4
High-density lipoprotein, fasting value ($mmol\,l^{-1}$)	128	1.1 ± 0.3	0.47–2.3

The reported values had been measured within a ±10-day range of the ciclosporin pharmacokinetic study that was carried out before transplantation.

between-run coefficients of variation for the assays were <7% for concentrations >30 $\mu g\,l^{-1}$. For quality control, the laboratory participates in the ciclosporin International Proficiency Testing Scheme.

Of the 166 patients who underwent the pharmacokinetic pretransplantation study, 162 were eligible to be included in the development of the population pharmacokinetic model (Table 1). The reasons for exclusion were: (i) <24 h between the oral and i.v. doses of ciclosporin; (ii) technical problems in the ciclosporin assay; (iii) technical problems with the ciclosporin infusion; and (iv) polycystic renal disease with severe hepatic involvement. All patients were on continuous ambulatory peritoneal dialysis or continuous cycling peritoneal dialysis, and those with congenital nephrotic syndrome had been nephrectomized. Intravenous data from all of the 162 children and oral data from the patients who received the microemulsion formulation ($n = 89$) were included in the study. Seventy-three patients received the conventional oral formulation and their oral data were excluded, because the conventional formulation is no longer used.

The ciclosporin concentration and covariate data were collected from patient records. Of the 162 patients who were included, 23% had one to four ciclosporin concentration data points missing.

Pharmacokinetic analysis

Population pharmacokinetic modelling was applied using NONMEM (version 5.1.1; GloboMax LLC, Hanover, MD, USA). All analyses were performed with the first-order conditional estimation method with interaction [22]. Graphical diagnostics, using the Xpose program [23] and comparison of competing models using the objective function values (OFV) in the likelihood ratio test, guided the model development. To obtain symmetric errors, the concentration data were log-transformed for the pharmacokinetic analysis.

Model development The structural model was established initially, followed by development of the covariate model. The stochastic model was refined interactively during the whole model development. The population model was initially developed for i.v. data, followed by inclusion of oral data and subsequent refinement of the model.

Defining the structural model involved evaluation of one-, two- and three-compartment models. The compartmental models were parameterized in terms of clearances and volumes with rate constants used to describe the absorption process. Both first-order and zero order absorption models, with and without lag time, were tried. In addition, an alternative absorption model appropriate to describe delayed absorption, the transit-compartment model [24], was tested. Models including a description of the saturable ciclosporin blood binding with an E_{max} model were also evaluated.

On the basis of i.v. data, possible covariate effects were evaluated for BW, height, body surface area (BSA) (calculated with the method of Gehan–George) [25], age, diagnosis, blood haemoglobin, haematocrit, serum creatinine, serum protein content, serum prealbumin, serum alanine aminotransferase (log-transformed due to skewed distribution), serum alkaline phosphatase, total plasma triglycerides, total plasma cholesterol, low-density lipoprotein and high-density lipoprotein. Missing covariate values were assigned the population median value.

First, BW was tried on all clearance and volume parameters one at a time, and on all of the clearance and

volume parameters at the same time. BW was included in the model using allometric scaling [26]. Second, on the basis of the derived model (including the allometric BW relation) and the observed individual concentrations, individual empirical Bayesian pharmacokinetic parameter estimates (EBE) were obtained within NONMEM. The EBE were plotted against the covariates for visual inspection [27]. The noncontinuous covariates that showed a correlation with a pharmacokinetic parameter and all continuous parameters were entered into the model and evaluated within NONMEM. The continuous covariates were modelled as a linear function, parameterized so that the relationship was centred on the median covariate value, here exemplified by the typical value of clearance (TVCL) and total plasma cholesterol (CHOL).

$$\mathrm{TVCL} = \theta_1 \times [1 + \theta_2 \times (\mathrm{CHOL} - \mathrm{median\ CHOL})] \quad (1)$$

In this case, θ_1 represents the estimate of clearance in a typical individual with median total plasma cholesterol, and θ_2 the fractional change in clearance with each unit change in total plasma cholesterol.

The covariate–parameter relationships were evaluated one at a time, using a common covariate effect, i.e. the same θ_2 (see Equation 1), for all volume and clearance parameters. The covariate–parameter relationships that produced a $\geq$10-point drop in the OFV (corresponding to a *P*-value of $\leq$0.001) when added simultaneously to all clearance and volume parameters were identified as important covariates. For such covariate–parameter relationships, a model allowing different covariate coefficients for clearance and volume parameters was evaluated.

A full model, including all the statistically significant covariate–parameter relationships, was assembled. Thereafter, covariate–parameter relationships were taken out of the model one at a time and only the relationships producing a $\geq$10-point increase in the OFV, when taken out, were left in the model.

Following establishment of the structural model also including the oral data, the covariate effects obtained with the i.v. model were challenged. Also, the EBE for the absorption constant (K_a) and oral bioavailability (F) were plotted against the covariates for visual inspection. Although no correlation was seen, BW was tested as a covariate on both K_a and F. After finalizing the model, other size covariates were tested instead of the allometrically scaled BW.

The stochastic model comprised evaluation of the residual error, interindividual variability (IIV) and interoccasion variability (IOV). The residual error was initially described with a slope-intercept model. The residual error model was also assessed for impact of the assay method and IIV on the magnitude of the residual error. An exponential model was used to describe the IIV in the pharmacokinetic parameters and covariance between individual parameters was assessed. IOV was modelled by an additional random effect as described previously [28].

Bootstrap The standard errors for the final population model parameters were obtained by a bootstrap method [29] in an automated fashion with the use of the programming library Perl-speaks-NONMEM (PsN) [30]. The bootstrap resampling was repeated 100 times, and the values of the parameters obtained were used to calculate relative standard errors for the final model parameter estimates.

Dose requirement calculations Dose requirement calculations were made to demonstrate the influence of the covariates on oral dosing. A target ciclosporin exposure [area under the concentration–time curve (AUC) of 2500 $\mu g\ l^{-1}\ h$, approximating the target AUC of a dose interval when ciclosporin is administered three times daily to stable kidney transplant patients [31]] was chosen. Doses were calculated on the basis of (i) the EBE estimates (true dose required), (ii) the population model and all covariate information and (iii) the population model and information on BW.

Results

Patient population

All patients (107 boys, 55 girls) had renal disease (congenital nephrosis of the Finnish type, $n = 64$; urethral valve, $n = 21$; polycystic renal disease, $n = 11$; nephronophtisis, $n = 11$; other diagnoses, $n = 55$) and were waiting for renal transplantation. One patient was of East African descent and the others were Finnish Whites. The median age at the time of the pretransplantation pharmacokinetic study was 3.8 years (range 0.36–17.5 years). Patient demographics are described in Table 1.

Structural and stochastic models

Two thousand four hundred and thirty-seven concentrations were available from the patients. A linear three-compartment model with first-order absorption without lag time best described the data. Interindividual variability (IIV) was assigned on all pharmacokinetic parameters. Based on a full variance–covariance matrix, the IIV model was simplified so that some parameters (the central and second peripheral compartment volumes and the corresponding intercompartmental clearance) shared

the same η, but allowing for different magnitudes of IIV (i.e. complete positive or negative correlation), whereas other parameters (total clearance, absorption rate constant and oral bioavailability) were not correlated at all. The addition of IOV to the model parameters did not improve the model.

The residual error was best described by the combination of a proportional and an additive component for i.v. administration and solely by a proportional component for oral administration. The addition of an IIV component to the residual error further improved the model. The effect of using two different quantification methods for ciclosporin concentrations was first tested in NONMEM by adding the assay method as a covariate on the clearance parameter. There was, however, no correlation between clearance and the assay method and this relationship proved to be nonsignificant. Second, the weighted residuals were plotted against time and a larger residual error was observed to be related to the old assay method than to the newer one. Hence, the influence of using two quantification methods for ciclosporin concentrations was best taken into account by introducing this factor as a covariate for residual error.

After initially finalizing the structural, covariate and stochastic models, the model produced some underprediction at the 15–24 h postdose time points following oral administration. Allowing different clearance parameters after oral and i.v. administration substantially corrected the underprediction. Hence, the final model was assigned a 23% smaller typical value of clearance after oral administration than after i.v. administration. Both the i.v. and oral population model predicted concentrations (PRED) are based on information about the covariate relationships and the typical population parameters, and thus left much variability unexplained when compared with the observed concentrations (DV) (Figure 1) or against time (Figure 2). However, the individual model predictions (IPRED), also containing information about the unexplained variability of the population parameters, displayed similar variability as did the observed concentrations (Figures 1 and 2).

Medication potentially interacting with ciclosporin

Eight patients were receiving medications potentially interacting with ciclosporin. One patient received carbamazepine during the study, two received oxcarbazepine and five received phenobarbital. To assess the effect of including these eight patients on the population parameters, they were deleted from the population dataset after the final model was estimated. The popula-

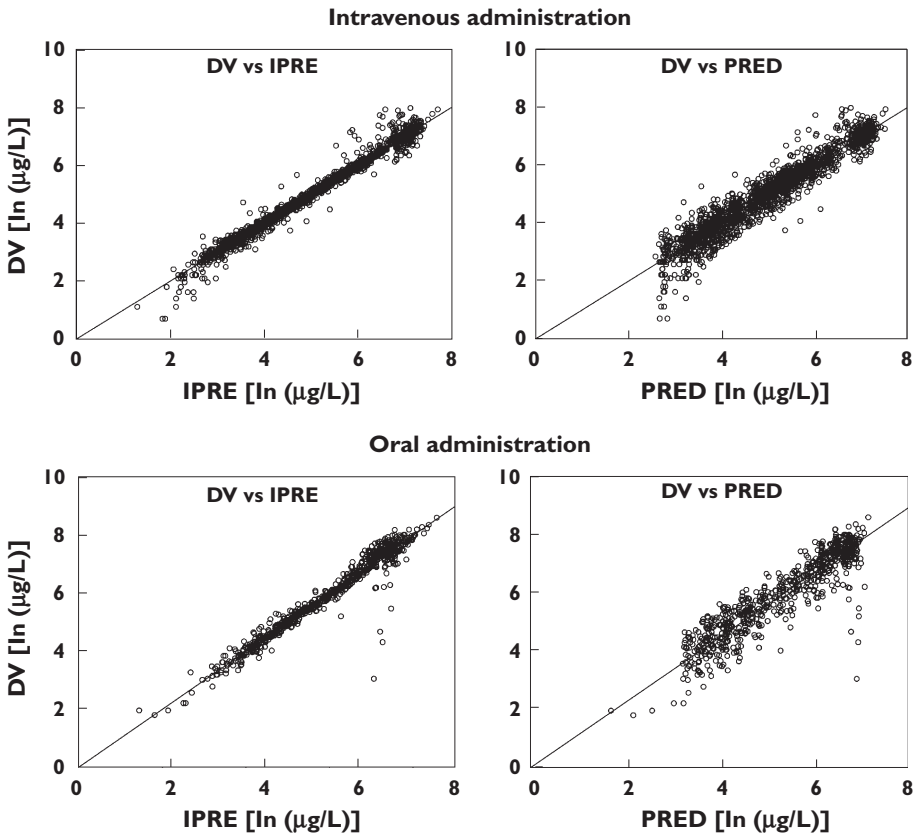

Figure 1

Observed ciclosporin concentrations (DV) *vs.* individual model predicted concentrations (IPRE) and population model predicted concentrations (PRED) with line of unity. Ciclosporin was given to 162 patients as an intravenous 4-h infusion (3 mg kg^{-1}) and a single oral dose (10 mg kg^{-1}) with an interval of at least 24 h between doses (usually 48–72 h). One patient was accidentally given only 1 mg kg^{-1} of ciclosporin orally

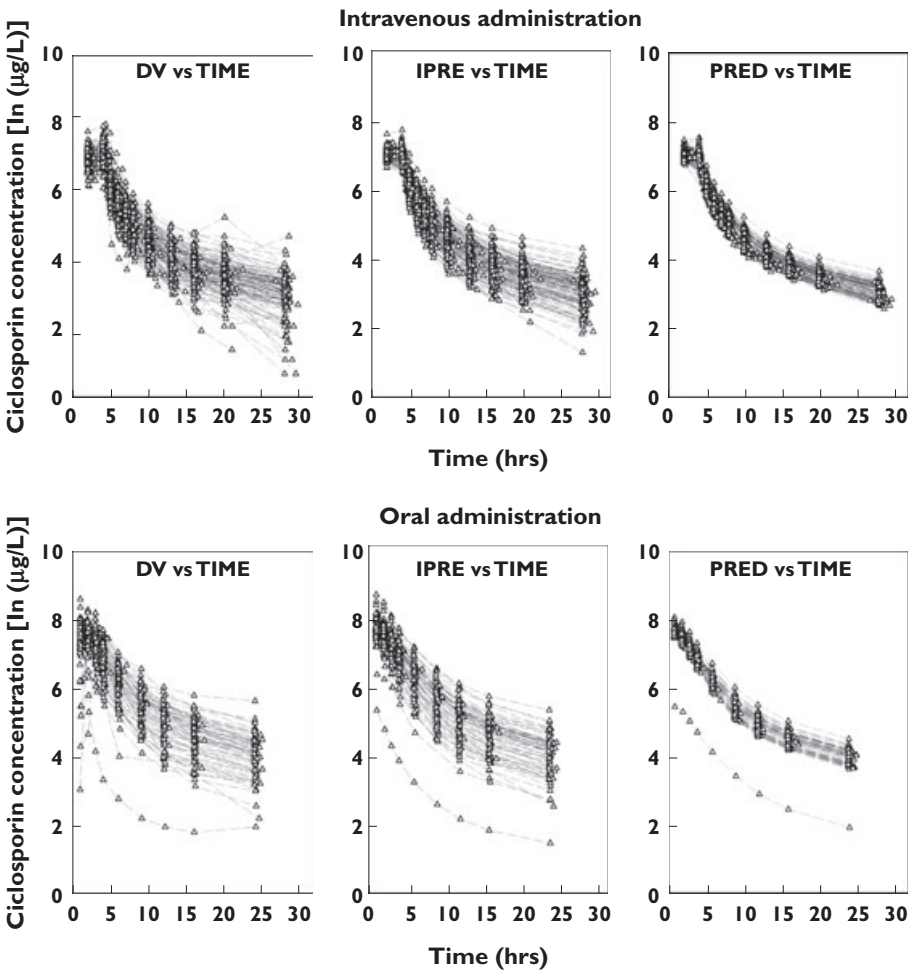

Figure 2
Observed ciclosporin concentrations (DV), individual model predicted concentrations (IPRE) and population model predicted concentrations (PRED) *vs.* time. Ciclosporin was given to 162 patients as an intravenous 4-h infusion (3 mg kg^{-1}) and a single oral dose (10 mg kg^{-1}) with an interval of at least 24 h between doses (usually 48–72 h). One patient (the outlier in the lower panel) was accidentally given only 1 mg kg^{-1} of ciclosporin orally

tion parameters were then estimated again, and virtually no changes were observed. Thus, the data from these patients were left in the final dataset. In the patients receiving phenobarbital, the clearance and oral bioavailability EBEs were in the 25th to 75th percentile range. The patient receiving carbamazepine and one of the patients receiving oxcarbazepine had clearance values in the highest quartile, whereas the other patient receiving oxcarbazepine had a clearance value in the lowest quartile.

Covariate model

In the final model, ciclosporin clearance was related to BW using an allometric model. Because the fixed, allometrically sound exponents (3/4 for clearance parameters and 1 for volume parameters) were very close to the BW exponents estimated using NONMEM (0.787 for clearance parameters and 0.951 for volume parameters), fixed values from the literature were chosen [26]. Competing models in which BSA, age or BW (without allometric scaling) were included as linear relations (one covariate coefficient for clearance parameters and one for volume parameters) were not superior, OFV being +8, +63 and −3 units, respectively, to the final model.

The other statistically significant covariates were haematocrit, plasma cholesterol and serum creatinine. For these covariates, the same covariate coefficients were assigned for all clearance and volume parameters, because different coefficients for these parameters did not significantly decrease the OFV. The same coefficients were also judged to be a rational choice, because these covariates were assumed to affect the unbound fraction of ciclosporin in blood and consequently influence the clearance and volume of distribution in parallel.

Pharmacokinetic parameters, covariate effects and developmental factors

The final population model parameters are presented in Table 2. The mean ± SD (range) individual empirical Bayesian pharmacokinetic parameter estimate (EBE) of

Table 2
Final parameter estimates and relative standard errors* (RSE)

Parameter	Parameter estimate	RSE (%)
Structural model parameters†		
CL (l h^{-1})	6.1	1.4
V_2 (l)	5.3	4.6
Q3 (l h^{-1})	1.5	2.8
V_3 (l)	19.6	4.6
Q4 (l h^{-1})	3.0	6.2
V_4 (l)	4.4	3.7
Cholesterol covariate effect	–0.054	23.2
Creatinine covariate effect	0.00021	26.3
Haematocrit covariate effect	–0.0073	34.1
Absorption rate constant (K_a) (1 h^{-1})	0.68	5.0
Oral bioavailability (F)	0.36	4.7
Inter-individual variability (IIV) parameters		
IIV CL (CV)	0.17	2.3
IIV V_2 (CV)	0.12	124.4‡
IIV Q3 (CV)	0.31	4.5
IIV V_3 (CV)	0.42	12.8
IIV Q4 (CV)	0.39	19.3
IIV V_4 (CV)	0.25	38.5‡
IIV K_a (CV)	0.33	8.0
IIV F (CV)	0.11	47.0
Residual error parameters		
Intravenous proportional error (CV)	0.089	5.7
Intravenous additive error (SD in μg l^{-1})	1.5	22.0
IIV intravenous residual error (CV)	0.43	9.1
Oral proportional error (CV)	0.2	7.4
IIV oral residual error (CV)	0.53	17.1
Analytical method covariate effect	2.6	11.3

**Relative standard error, Standard error of parameter estimate/parameter estimate × 100%. †CL, Typical value of clearance; V2, typical value of the central compartment volume; Q3, typical value of the 1st intercompartmental clearance; V3, typical value of the 1st peripheral compartment volume; Q4, typical value of the 2nd intercompartmental clearance; V4, typical value of the 2nd peripheral compartment volume. The typical values refer to a patient with a body weight of 13 kg, cholesterol of 5.4 mmol l^{-1}, serum creatinine of 524 μmol l^{-1} and a haematocrit of 31%, according to the following covariate model: Typical value = Typical parameter estimate × (Body weight/13)^A × [1 – 0.0542* × (Cholesterol – 5.4)] × [1 – 0.00732 × (Haematocrit – 31)] × [1 + 0.000214 × (Serum creatinine – 524)], A = 3/4 for clearance parameters and A = 1 for volume parameters. ‡Relative standard error for the corresponding scaling factor (for V2 and V4).*

body weight normalized clearance (CL/BW) was 0.44 ± 0.09 l h^{-1} kg^{-1} (0.19–0.73 l h^{-1} kg^{-1}), the EBE of the body weight normalized volume of distribution (V/BW) was 2.35 ± 0.65 l kg^{-1} (1.26–4.61 l kg^{-1}) and that of the oral bioavailability (F) was 36 ± 8% (10–60%) (Figure 3A,D,E).

The CL/BW decreased with increasing age after the second year of life (Figure 3A, Table 3). The CL/BW was 0.48 ± 0.09 l h^{-1} kg^{-1} (0.30–0.73 l h^{-1} kg^{-1}) in prepubertal children (≤8 years) and 0.38 ± 0.07 l h^{-1} kg^{-1} (0.19–0.64 l h^{-1} kg^{-1}) in older children. Very young patients (0.36–1 year, $n = 36$) had a similar CL/BW [0.47 ± 0.08 l h^{-1} kg^{-1} (0.33–0.67 l h^{-1} kg^{-1})] to other prepubertal children. Normalization of clearance for allometric BW removed the relationship between age and clearance, whereas clearance/BSA showed an increase with age (Figure 3B,C). In accordance with this finding, the estimates of liver volume, obtained with the meta-analysis-based formula of Johnson *et al.* [32], could also be described by an allometric weight model with an exponent of 3/4 in our population (Figure 4). No trends could be observed when examining the relationship between F or V/BW and age (Figure 3D,E). Allometrically scaled BW was the covariate most influential on ciclosporin clearance and volume parameters, with four- and sixfold differences between individuals accountable to BW (Figure 5). The other covariates had smaller contributions to the interindividual differences in clearance and volume parameters, but as large as 20–30% differences between individuals could be attributed to changes in serum creatinine, haematocrit and total plasma cholesterol (Figure 5).

The estimated oral BW-adjusted dose requirements (mg kg^{-1}) of ciclosporin varied greatly between individual patients (Figure 6). The dose requirement showed a similar relationship to age as CL/BW, with the highest doses required in prepubertal children (≤8 years). The full covariate model predicted the individual oral dose requirement with a slightly smaller prediction error [3 ± 35% (–74 to 73%)] than a model including BW as the only covariate [7 ± 36% (–65% to 122%)] (Figure 7).

Discussion

In our population of 162 infants, children and adolescents, aged 0.36–17.5 years, a three-compartment model with BW (scaled allometrically), haematocrit, plasma cholesterol and serum creatinine as covariates for clearance and volume parameters best described the pharmacokinetics of ciclosporin. Accordingly, allometrically scaled BW alone was responsible for fourfold differences in uncorrected ciclosporin clearance and sixfold

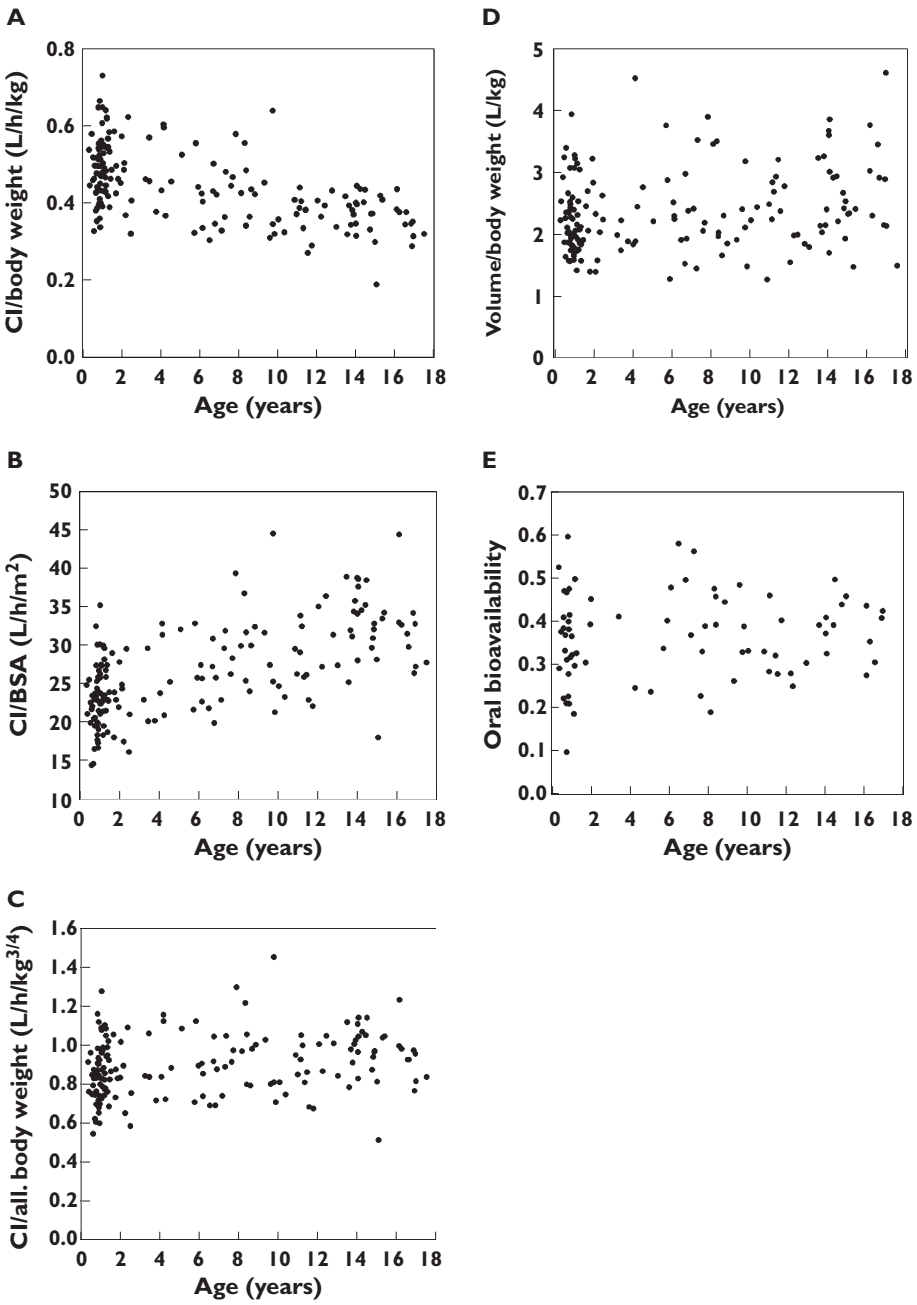

Figure 3
Individual empirical Bayesian estimates of ciclosporin clearance (**A,B,C**), volume of distribution (**D**) and oral bioavailability (**E**) plotted against age in 162 paediatric renal transplant candidates. BSA, Body surface area; All. body weight, allometrically scaled body weight

Table 3
Body weight-normalized mean ± SD (range) individual empirical Bayesian estimates of ciclosporin clearance (CL/BW), volume of distribution (*V*/BW) and oral bioavailability (*F*) grouped by age in 162 paediatric renal transplant candidates

Age group	*n*	CL/BW	*n*	*V*/BW	*n*	*F*
4–8 months	9	0.48 ± 0.07 (0.33–0.58)	9	2.49 ± 0.59 (1.63–3.39)	7	0.38 ± 0.10 (0.22–0.52)
8–12 months	27	0.47 ± 0.09 (0.34–0.66)	27	2.21 ± 0.50 (1.56–3.94)	16	0.34 ± 0.12 (0.10–0.60)
1–2 years	33	0.51 ± 0.08 (0.39–0.73)	33	2.16 ± 0.57 (1.39–3.28)	8	0.34 ± 0.09 (0.19–0.50)
2–8 years	34	0.45 ± 0.09 (0.30–0.62)	34	2.34 ± 0.73 (1.27–4.52)	14	0.39 ± 0.11 (0.23–0.58)
8–12 years	23	0.39 ± 0.09 (0.27–0.64)	23	2.40 ± 0.60 (1.26–3.5)	16	0.36 ± 0.09 (0.19–0.48)
12–18 years	37	0.37 ± 0.05 (0.19–0.44)	37	2.57 ± 0.74 (1.47–4.61)	17	0.37 ± 0.07 (0.25–0.50)

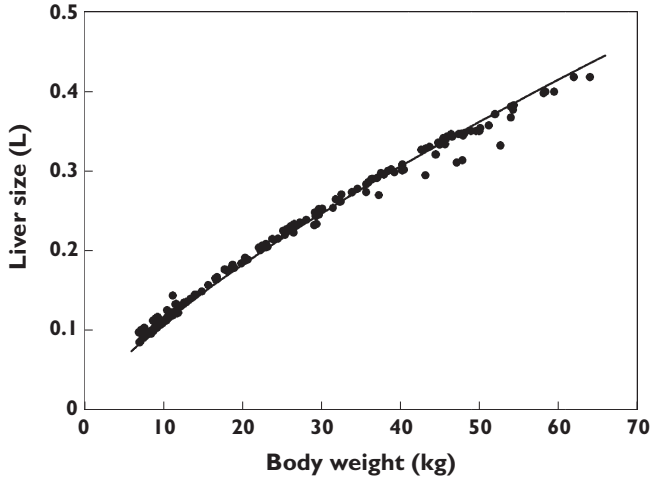

Figure 4
Relationship between body weight and liver size, demonstrated here by the individual liver sizes (●) in our 162 patients calculated with the formula proposed by Johnson *et al.* [32]: liver volume = $0.722 \times BSA^{1.176}$; and the allometrically calculated liver size (dark line) based on the data obtained by the previous formula and allometric principles [26]: liver volume = $0.46 \times$ (body weight of child per 70 kg)$^{0.75}$

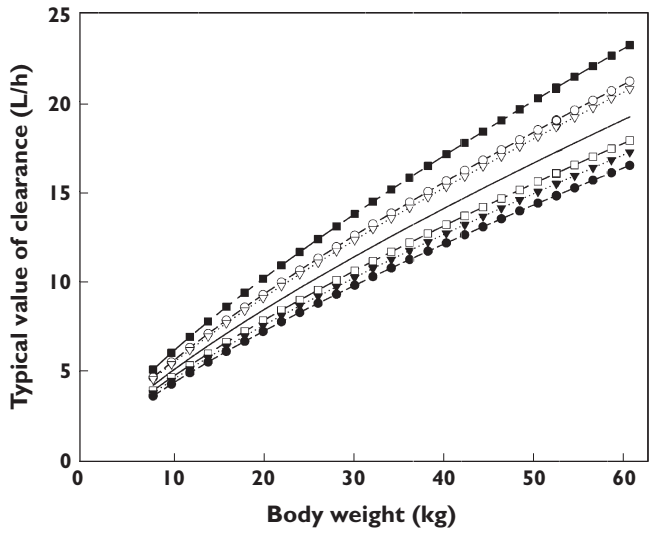

Figure 5
The magnitude of the covariate effects. The typical value of clearance is presented as a function of body weight, given median values of the other covariates in the model. {The equation for the typical value of clearance used in the population pharmacokinetic model and in depicting the magnitude of the covariate effects: typical value of clearance = $6.08 \times [\text{body weight}/13]^{3/4} \times [1 - 0.0542 \times (\text{cholesterol} - 5.4)] \times [1 - 0.00732 \times (\text{haematocrit} - 31)] \times [1 + 0.000214 \times (\text{serum creatinine} - 524)]$.} The influence of the other covariates is presented at extreme values according to the covariate value range in the present study. Typical value of clearance, (—); Cholesterol 3.5 mmol/L, (○); Cholesterol 8 mmol/L, (●); Creatinine 200 μmol/L, (□); Creatinine 1500 μmol/L, (■); Haematocrit 20%, (▽); Haematocrit 45%, (▼)

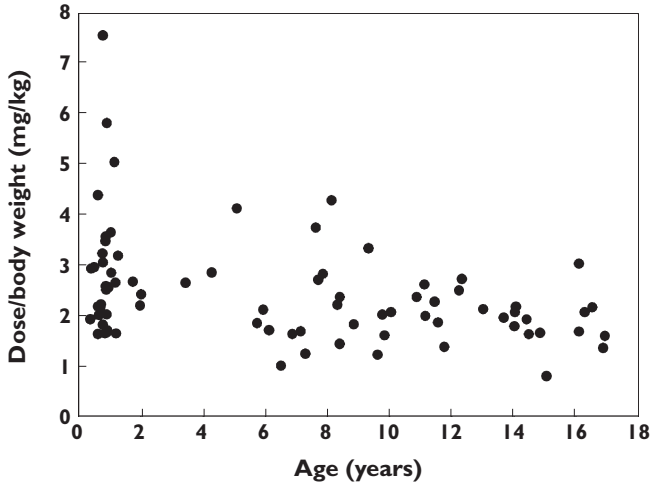

Figure 6
Oral dose requirements based on the individual Bayesian estimates (EBE) of clearance and oral bioavailability aiming at a ciclosporin AUC of 2500 μg l^{-1} h. The oral doses were calculated as follows: dose requirement = AUC × EBE of clearance/EBE of oral bioavailability

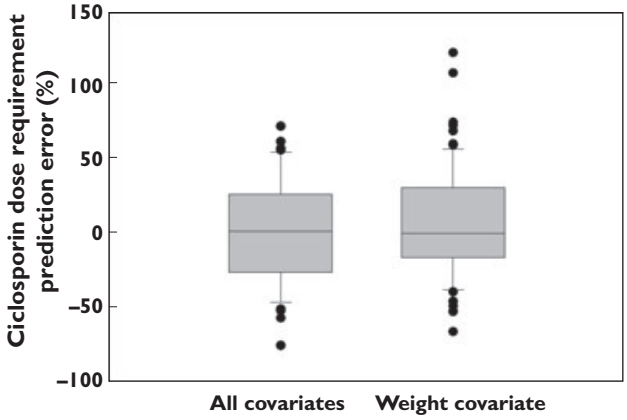

Figure 7
Box and whisker plots of the prediction errors for oral dose requirements calculated using the population model and (**A**) all covariate information (body weight, serum creatinine, haematocrit and total plasma cholesterol) in the population model and (**B**) the body weight covariate alone to achieve a ciclosporin exposure of 2500 μg l^{-1} h. The oral doses were calculated as follows: (**A**) dose requirement by all covariates = AUC × Typical value of clearance (including all covariates)/Typical value of oral bioavailability; (**B**) dose requirement by body weight = AUC × Typical value of clearance (including the body weight covariate only setting the other covariates to median values)/Typical value of oral bioavailability. Relative prediction error (%) = (calculated dose – true dose requirement*)/true dose requirement × 100%. The whiskers above and below indicate the 10th and 90th percentiles, the boundaries of the box indicate the 25th and 75th percentiles, the line in the middle indicates the median value and the outliers are plotted separately. *True dose requirement = AUC × individual Bayesian estimate of clearance/individual Bayesian estimate of oral bioavailability

differences in volume parameters. In addition, serum creatinine, haematocrit and total plasma cholesterol explained interindividual differences in clearance (Figure 5) and volume of distribution of 20–30%. These findings have implications for understanding the effects of age and body size on the pharmacokinetics of ciclosporin and for the individualization of ciclosporin dosing in children. The findings also improve our understanding of the development of CYP3A4-mediated metabolism in general.

In our study, the range of BW-normalized ciclosporin clearance values extended to higher values (0.19–0.73 l h^{-1} kg^{-1}) than those previously reported for adults (0.2–0.5 l h^{-1} kg^{-1}). The volume of distribution was similar or slightly smaller (1.26–4.61 l kg^{-1}) than in adults (2–11 l kg^{-1}). However, the oral bioavailability (10–60%) was within the range of adult values [33–36].

The development of validated paediatric pharmacokinetic models is often difficult due to lack of sufficient pharmacokinetic data, especially for the 0–5-year age range. This holds true for ciclosporin as well as for other CYP3A4 substrates. Although it has been proposed that small children generally have a higher ciclosporin CL/BW than do adults, few studies have documented this [9]. Moreover, few bioavailability studies have been conducted in the paediatric population with the microemulsion formulation of ciclosporin [9, 10]. Published data concerning young children (≤5 years) have been limited, and virtually no data have existed concerning patients aged <1 year. Our prepubertal children (≤8 years) had approximately 25% higher CL/BW than did older children (Figure 3A) or adults [33–36]. The CL/BW was the highest in children between 4 months to 2 years of age and declined steadily thereafter, reaching adult values by about 12 years (Figure 3A). However, *F* was similar in all age groups (Figure 3E), which implies that younger (0–8 years) children need around 25% larger daily ciclosporin doses than do older children (≥8–18 years) (Figure 4). Moreover, because the *V*/BW did not change with age (Figure 3D), it can be estimated that the half-life of ciclosporin is about 20% shorter in younger than in older patients. To compensate for the rapid elimination in prepubertal children (≤8 years), ciclosporin dosing can be increased from twice daily dosing by administering a third daily dose, to achieve drug exposures (average daily concentrations) similar to those in older patients with slower elimination [10, 31].

In the paediatric population, pharmacokinetic differences due to age-related factors cannot always be distinguished from differences due to factors related to size, because the effect of body size may mask the effects of other covariates (especially in a population with both young/small and older/larger children). To identify covariates other than body size, such as genotypic or demographic characteristics, it is thus desirable to standardize or adjust these parameters to an appropriate body size measure, e.g. by allometric relationships [37, 38]. BSA and normal BW are size covariates that are easy to use in a clinical setting. However, allometric BW scaling can produce more accurate estimates, which is a valuable aid in a model-building environment when the goal is to identify covariate effects. Our data have shown that young children have a higher CL/BW and lower CL/BSA than older children (Figure 3A,B). With the allometric BW relationship, however, no clear correlation could be seen between age and clearance/allometric BW (Figure 3C).

CYP3A4 constitutes approximately 30–40% of the total hepatic amount of cytochrome P450 enzymes and metabolizes many exogenous and endogenous compounds, including ciclosporin [39, 40]. Data on the ontogeny of CYP3A4 are scarce [19, 20]. According to one study, the activity of CYP3A4 is extremely weak or absent in fetal liver samples and begins to rise after birth to reach 30–40% of adult activity after 1 month [41]. It is not known exactly when the activity attains adult levels, although previous data suggest that older infants (from 1 year onwards) and children have similar hepatic CYP3A4 activity (per gram of liver) to that of adults [20, 41, 42]. Liver size shows an approximately allometric relationship to BW, having an exponent around 0.7–0.9 (Figure 4) and it has been suggested that liver blood flow is approximately proportional to liver size in children [43]. This, together with a constant CYP3A4 activity (per gram of liver) with increasing age, could explain why allometric scaling with $BW^{3/4}$ well described the relationship between ciclosporin clearance and BW in our paediatric patient population (age range 0.36–17.5 years). The same scaling has produced relatively good results with the limited data available for midazolam and alfentanil, other typical CYP3A4 substrate drugs [20].

In paediatric drug development, it is usually difficult to perform dose escalation studies similar to those carried out in adult patients. An initial estimate of an appropriate dose may therefore be obtained via extrapolation approaches carried out on the basis of adult pharmacokinetic data. It is difficult to determine *a priori* which scaling method is the best for a given drug, but some evidence supports the use of $BW^{3/4}$ for the scaling of clearance from adult values [44, 45]. Therefore, and because the allometric power estimate that we estimated for BW in NONMEM was very close to 3/4, it was decided to use this fixed exponent. Thus, these results

suggest that the fixed 3/4 exponent can be used for the modelling of clearance of CYP3A4 substrates, at least for children >6 months old.

The finding that the oral bioavailability of ciclosporin is independent of age is consistent with a relatively constant hepatic CYP3A4 activity across age groups. Moreover, it could be speculated that the fraction of the first-pass metabolism of ciclosporin occurring in the small intestine by the interplay of CYP3A4 and P-glycoprotein is constant with age. Although no factors covaried with the oral bioavailability of ciclosporin, the bioavailability ranged from 10% to 60%, and its IIV was 11%. Factors such as dietary constituents and genetic polymorphisms affecting the membrane transport and metabolism of ciclosporin could be possible causes of the IIV in its oral bioavailability. For example, Hesselink *et al.* [36] have found that renal transplant recipients carrying the *CYP3A4* g.392A→G variant allele have a 9% larger oral ciclosporin clearance than do noncarriers.

Ciclosporin is extensively bound to plasma lipoproteins and red blood cells [3]. As the clearance and volume of distribution of ciclosporin can be expected to increase with an increasing unbound fraction, it is logical that plasma cholesterol and haematocrit values were significant covariates for both the clearance and volume of distribution of ciclosporin. These parameters were in inverse relation to cholesterol and haematocrit values. Furthermore, in our population pharmacokinetic model, the typical values for clearance and volumes of distribution increased with rising serum creatinine. A possible explanation is that serum creatinine acts as a marker for factors that reduce ciclosporin blood binding or induce its metabolism, or both [10, 14, 46]. In addition to the covariate relationships found in our study in paediatric patients, serum bilirubin, for example, has been found to be a significant covariate for oral clearance in adult renal transplant recipients [33].

The observation that the clearance of ciclosporin would be about 20% smaller after oral than after i.v. administration is obviously an artefact. Because more metabolites are formed after oral administration of ciclosporin [47], and because the antibody of the specific RIA method can cross-react slightly with ciclosporin metabolites, oral administration of ciclosporin can result in somewhat higher concentrations than does i.v. administration [48, 49]. Although this is a problem that cannot be overcome entirely by modelling, the model predictions, especially the population estimate and the EBE of oral bioavailability, can be improved by allowing clearance to differ after i.v. and after oral administration. The use of two slightly different analytical methods is also a point of concern that could possibly cause bias in the estimates. The residual error with the old analytical method was approximately 2.5 times larger than with the newer method. However, we believe that by adding analytical method as a covariate to the residual error, the problem could be adequately controlled.

Generalization of our pharmacokinetic results to the post-transplantation situation should be made cautiously, because the pharmacokinetics of ciclosporin may change after renal transplantation [50–52]. However, the covariate effects and age-dependent changes seen in ciclosporin pharmacokinetics prior to transplantation are likely to remain also after renal transplantation. In our paediatric population, the mean serum creatinine value decreased by 85% (pretransplantation range 165–1669 μmol l^{-1}, 5–95th percentile post-transplantation range 20–200 μmol l^{-1}) after transplantation (time after transplantation 1 week to 7 years). The ranges for haematocrit (pretransplantation 15–48%, post-transplantation 17–57%) and total plasma cholesterol (pretransplantation 3.1–9.8 mmol l^{-1}, post-transplantation 2.9–8.1 mmol l^{-1}) remained virtually the same, although less anaemia occurred after transplantation. Because only serum creatinine changed markedly after transplantation, covariate-dependent changes in ciclosporin pharmacokinetics due to transplantation are not likely to be extensive. It is possible, however, that the exact predictions of dose requirements made in the present study will not hold true for very long after renal transplantation, but can be applied if ciclosporin treatment is initiated prior to the transplantation operation. In future, our model can be further refined by inclusion of pharmacokinetic data from post-transplant patients in developing the model. Thereafter, the model can be implemented into a Bayesian dosing programme and used to predict individual starting doses of ciclosporin in paediatric renal transplant candidates prior to transplantation.

In conclusion, BW, serum creatinine, haematocrit and total plasma cholesterol affected ciclosporin pharmacokinetics in paediatric renal transplant candidates. With increasing BW and serum creatinine, ciclosporin clearance and volume parameters increased. With increasing plasma cholesterol and haematocrit, clearance and volume parameters decreased. Allometrically scaled BW was a sufficient size covariate – age was not necessary in the model as an additional covariate. A 25% higher CL/BW of ciclosporin could be seen in prepubertal children (≤8 years) than in older patients, while no age-related changes in the *V*/BW or in oral bioavailability were observed. This implies that prepubertal children (≤8 years) need a larger daily dose/BW, and perhaps a shorter dose interval than older children when aiming

at similar ciclosporin exposure. Finally, after adult CYP3A4 activity is reached (in the range of 6–12 months of age), allometrically scaled BW seems to be a good predictor of the hepatic clearance for a CYP3A4 substrate such as ciclosporin.

This work was supported by a grant from the Finnish Kidney Foundation and by the Helsinki University Central Hospital Research Fund (Helsinki, Finland).

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