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RESEARCH PAPER

Influence of Dosing Schedule on Organ Exposure to Cyclosporin in Pediatric Hematopoietic Stem Cell Transplantation: Analysis with a PBPK Model

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ABSTRACT

Purpose Cyclosporin is administered by intermittent infusions (II) or continuous infusions (CI) to prevent acute graft-*versus*-host disease (aGVHD). Because cyclosporin disposition is nonlinear, organ exposure may be higher after II than after CI, but saturation of receptors must be accounted for. The aim of the study was to compare both types of administration using a mechanistic model.

Methods A physiologically based pharmacokinetic model was developed to estimate cyclosporin exposure and receptor occupancies (RO) in aGVHD target organs and kidneys and to compare these estimations in pediatric patients that received cyclosporin either by II or CI. The relevant biological parameters were based on a clinical study in 2 groups of pediatric patients that received cyclosporin either by II ($n = 31$) or CI ($n = 30$).

Results Simulations showed that the exposure to cyclosporin in the interstitial fluid of aGVHD target organs was greater at day 1 after II than after CI. In kidneys, the opposite order was observed. AUC_{RO} in all organs was greater after CI than after II. The therapeutic index (the ratio of AUC_{RO} in blood to AUC_{RO} in kidneys) was greater with CI than with II.

Conclusions CI may be slightly more favorable than II for aGVHD prevention.

KEY WORDS cyclosporin · GVHD · hematopoietic stem cell transplantation · PBPK modelling

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INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is curative in several malignant and non-malignant haematological diseases, inborn errors of metabolism and immunodeficiencies. The major complication of allogeneic HSCT is graft-*versus*-host disease (GVHD) (1). Despite progress in prophylaxis, acute GVHD (aGVHD) is still responsible for significant morbidity and mortality (2). aGVHD mainly occurs in three organs: skin, intestines and liver (3).

Cyclosporin, an immunosuppressant drug belonging to the family of calcineurin inhibitor, is used to prevent aGVHD. Cyclosporin acts on T-lymphocytes, which circulate in blood and lymph. The mechanism of action of cyclosporin is the inhibition of the interleukin-2 (IL-2)-driven proliferation and activation of T-lymphocytes. By impairing IL-2 production by T-helper cells, the drug suppresses proliferation and generation of T-lymphocytes while sparing T-suppressor cell subpopulation. Cyclosporin exhibits a relatively narrow therapeutic window (4) and large inter-individual pharmacokinetic variability. Low exposure to immunosuppressant drugs is one of the risk

factors for developing aGVHD. In the early phase following HSCT, it is important to determine the optimal dose of cyclosporin to achieve and maintain the target blood concentration because of the important link between cyclosporin exposure and aGVHD incidence: a negative relationship was found (5–11). A positive impact of a monitoring cyclosporin regimen on aGVHD incidence and outcome was also demonstrated (12,13).

There is no consensus on the appropriate administration route for cyclosporin in the prevention of GVHD; some centers implement one or two intermittent infusions, and others use 24 h continuous infusions (14). Furthermore, there is no consensus on the optimal time to measure cyclosporin blood concentrations or the appropriate target concentrations (5,15). Finally, cyclosporin distribution is diffusion-limited and exhibits several sources of non-linearity, due to saturable binding to red blood cells and to cellular components in most organs (16). There are two consequences of non-linearity. First, given a constant total daily dose, the steady-state exposure of T-lymphocytes to cyclosporin in organs may depend on the rate of infusion. Thus, intermittent and continuous infusion may not be equivalent in terms of efficacy and safety. Second, the ratio of steady-state blood cyclosporin concentration to its concentration in the interstitial space of target organs may depend on the rate of infusion. Thus, the target blood concentrations may depend on the dosing schedule. In theory, it should be possible to address these questions with randomized clinical trials that compare the modes of administration and/or the target concentrations. However, in practice, patients with HSCT frequently require adjustments in the cyclosporin dose and/or target concentration during the early phase; thus, the effect to be studied is confounded, and comparisons are difficult or impossible.

Rowland and co-workers proposed a physiologically based pharmacokinetic (PBPK) model of cyclosporin based on rat physiology that takes into account the various sources of non-linearity (17). This kind of model offers the ability to simulate the kinetics of the drug in several sub-compartments of the main organs. Thus, it may facilitate our understanding of the effect of dosing schedule on exposure and the relationship between exposure in the blood and that in the organs.

In the present work, this previously published PBPK (17) model was used in order to simulate cyclosporin exposure in the target organs of aGVHD, to compare the exposures and receptor occupancies of pediatric patients that received cyclosporin either by intermittent or continuous infusion, and to explore the influence of several factors on these parameters. The relevant biological parameters and their interindividual variability are estimated using a clinical study in pediatric patients.

MATERIALS AND METHODS

Clinical Study

This was a retrospective, monocentric study. We studied the records of pediatric patients (4 m to 17 y) that underwent an allogeneic hematopoietic stem cell transplantation and received cyclosporin for the prevention of graft-*versus*-host disease. The patients gave written consent to use their data at the time of admission. Cyclosporin treatment started the day before transplantation. The patients comprised two groups. In the first group, 30 patients were treated with cyclosporin administered by continuous infusion (CI group). In the second group, 31 patients received cyclosporin by intermittent infusion, twice daily (for 2 h every 12 h; II group). The patients in the two groups were matched regarding age, sex, indication for transplantation (malignant *versus* non-malignant disease), proportion of related donors, administration of anti-lymphocyte serum, and number of mismatches when the donor was unrelated. The stem cell source was unmanipulated bone marrow from all donors. No methotrexate was administered.

For each patient, periodic monitoring of whole blood cyclosporin concentration was performed (EMIT; Dade Behring) on a Cobas Mira automate (18). The dosing regimen was adjusted with a Bayesian method (USC*-Pack® Software, version 10.0, Laboratory of Applied Pharmacokinetics, University of Southern California, USA) to achieve and maintain the target blood cyclosporin concentration. The target concentrations were defined as described in our previous studies (5,6). In the CI group, the target steady-state concentration was 200 μg/l (malignant disease) or 280 μg/l (non-malignant disease). In the II group, the target was a 12 h-trough concentration of 110 μg/l (malignant disease) or 130 μg/l (non-malignant disease).

The severity of aGVHD was classified according to the Glucksberg classification. This criteria include a rating from grade 0 (no GVHD) to grade IV (maximum severity). Grades are determined by a combination of different organ scores and a reduction in clinical performance. The organs (skin, intestines, and liver) are scored independently based on a 1 to 4 scale that depends on the degrees of skin rash, diarrhoea volume, and total bilirubin concentrations, respectively.

PBPK Model Of Cyclosporin Disposition

PBPK Model of the Rat

Global PBPK Model Framework. The physiologically based pharmacokinetics (PBPK) model describes drug disposition

in the blood and in various organs and tissues. Our PBPK model is close to the one proposed by the Rowland *et al.* team, developed in rats (17). Our global PBPK model comprised 11 organs (lung, heart, kidney, bone, muscle, spleen, liver, intestines, adipose tissue, and skin) and the corresponding arterial and venous pools. It assumed that cyclosporin transport occurs solely by blood flow.

Physiological Parameters. The physiological parameter values (organ volumes and blood flow rates) and fractions of vascular and interstitial spaces in rats were derived from the literature (17,19,20). The total bone volume included cortical and trabecular bone, red and yellow marrow, cartilage, and periarticular tissue. Marrow represented 29% of the total bone volume (21).

Blood Distribution. First, the distribution of cyclosporin in the blood is governed by its binding to plasma proteins and red blood cells. In plasma, cyclosporin is highly bound, principally to lipoproteins (95% of the total protein binding). The unbound fraction of plasma cyclosporin (fu_P) was 6%(19), and this value was independent of the cyclosporin concentration over the range of 20 to 20000 μg/l (22). Second, there is a single specific binding site on or in red blood cells, characterized by a dissociation constant ($K_{D,BC}$=185 μg/l, expressed as unbound concentration) and a binding capacity (nP_T=4640 μg-eq./l)(20). Finally, passive diffusion into blood cells is characterized by a permeability-surface-area product (PS_{BC}=0.56 l/h per l of blood)(20). As a result, the cyclosporin distribution is influenced by the hematocrit.

Interstitial Space Distribution. The distribution of cyclosporin in the interstitial space is governed by instantaneous, non-saturable binding to lipoproteins. To estimate the interstitial unbound fraction of cyclosporin (fu_I), we used the ratio of lipoprotein concentrations between the plasma and the interstitial space (IPR). The ratio for each organ was based on values from the literature (23).

Tissue Distribution. The exchange between interstitial and intracellular spaces was characterized by a permeability-surface-area product of the tissue cells (PS_{TC}). The value of PS_{TC} varied among organs and was based on previous estimates for each organ (17). In tissue cells, intracellular binding was characterized by the unbound fraction of tissue cyclosporin (fu_{TC}).

In all organs except liver, kidney, and lung, cyclosporin distribution was limited by diffusion. For the three remaining organs, distribution was limited by blood flow, and PS_{TC} was fixed at 30 times the organ blood flow.

The local model described the kinetics of distribution in the cells of each organ. Cyclosporin may bind to intracellular components (saturable, nonlinear process) or equilibrate with a deep-pool (non-saturable, linear process) (Fig. 1)(17). The most appropriate model was chosen for each organ: muscle and adipose tissue were well described with a linear tissue distribution, and the other organs were more appropriately described with nonlinear tissue distributions (16). For the linear distribution model, the kinetics of equilibrium was characterized by an association flux (k_{ass}) and a dissociation flux (k_{dis}). Cyclosporin bound to this site was called the "deep pool." For the nonlinear distribution model, intracellular binding was saturable (B_T is the number of binding sites) with instantaneous equilibrium characterized by a dissociation constant ($K_{D,TC}$). The values for these tissue distribution parameters are shown in Table I.

The equations for the distribution of cyclosporin in the blood, interstitial, and intracellular spaces of tissues were previously described by Kawai and Rowland (17).

Clearance. The excretion of cyclosporin in urine is negligible (24). Cyclosporin metabolism and elimination principally occur in the liver, by cytochrome P-450 (CYP) 3A4 and 3A5(25). In this model, it was assumed that elimination occurred only in the liver (19). Consequently, blood clearance (CL_B) was equal to hepatic clearance (CL_H). Hepatic clearance was related to fu_B (unbound fraction of cyclosporin in the blood), Q_H (hepatic blood flow), and

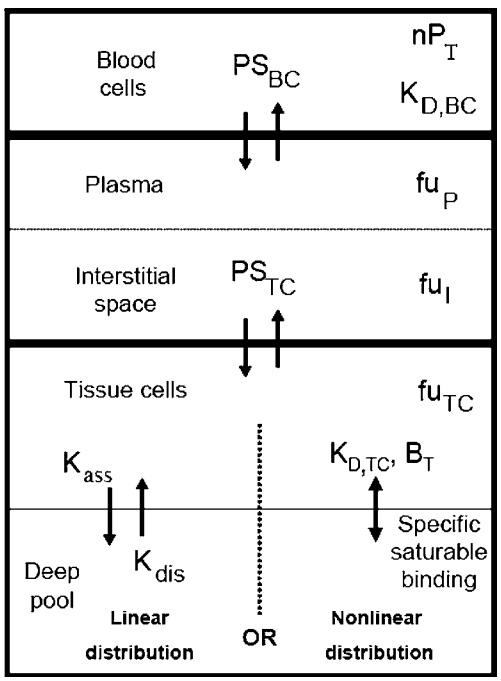

Fig. 1 Schematic representation of local model for linear tissue distribution and nonlinear tissue distribution.

Table I Values of Tissue Distribution Parameters for Linear and Nonlinear Tissue Distribution in Rats (16,18)

Linear tissue distribution				
Organs	PS_{TC} (l/h)	fu_{TC}	k_{ass} (l/h)	k_{dis} (l/h)
Muscle	2.4	0.041	0.00055	0.000026
Adipose tissue	0.079	0.001	1.2	0.0144
Nonlinear tissue distribution				
	PS_{TC} (l/h)	fu_{TC}	B_T (μg-eq/l)	$K_{D.TC}$ (μg/l)
Lungs	High[a]	0.015	4800	0.7
Heart	0.14	0.017	1900	0.2
Bone	0.39	0.1	6000	60.0
Skin	0.3	0.143	8500	39.0
Thymus	0.007	0.1002	10000	15.0
Kidney	High[a]	0.013	6300	5.8
Spleen	0.074	0.01	7800	10.0
Liver	High[a]	0.009	14000	16.0
Intestines	0.039	0.021	2400	4.8

[a] PS_{TC} was set at 30 times the blood flow in the organ

CL_{int} (hepatic intrinsic clearance of cyclosporin) in a well-stirred model. Furthermore, the hepatic intrinsic clearance was assumed to vary with the unbound intracellular cyclosporin concentration in the liver, according to a Michaelis Menten model, characterized by Km (concentration for half maximal rate: 600 µg/l) and Vmax (maximum rate of metabolism: 577 µg/h).

Validation of the PBPK Model in Rats. Our PBPK model of the rat was implemented in ADAPT II® software (26). The physiological and cyclosporin distribution parameters were fixed to their typical values. The hematocrit was fixed at 0.4 (19). Cyclosporin kinetics for a 2 min intravenous infusion of 6 mg/kg was simulated. The simulated cyclosporin concentration profiles in the blood and in the 11 organs were compared with those obtained experimentally by the Rowland team (17).

Scale-Up for the Adult Human Model

Physiological Parameters. The PBPK model of the rat was scaled up to represent an adult human by replacing rat organ volumes and blood flow values with those of a standard adult human (70 kg body weight) (17). The fractions of vascular and interstitial spaces were assumed to be equal in rats and humans (Table II).

Cyclosporin Distribution Parameters. Rat and human distribution parameters were assumed to be the same, including the values of fu_P, $K_{D,BC}$, nP_T, lipoprotein concentration ratios, fu_{TC}, and fu_I. Values of k_{ass}, k_{dis}, and PS_{TC} in rats were multiplied by the ratio of human to rat body weight. B_T and $K_{D,TC}$ were assumed to be identical per unit of organ mass (17).

Clearance. For the adult human model, the hepatic intrinsic clearance was set to 1470 l/h.

Pediatric Population Adaptation

Physiological Parameters. The physiological organ volumes in children (V_{child}) were obtained by correcting the adult values by the ratio of body weights (BW) for children and a standard adult of 70 kg (27):

$$V_{child} = V_{adult} \times \frac{BW_{child}}{BW_{adult}}$$

The fractions of interstitial and vascular space were derived from Bjorkman (28). The value for bone was taken from Edginton (29), and the value for the adult thymus was used for children.

The values of organ blood flow (Q) were derived from the values of the standard adult according to the following allometric equation (27):

$$Q_{child} = Q_{adult} \times \left(\frac{BW_{child}}{BW_{adult}}\right)^{\frac{3}{4}}$$

Cyclosporin Distribution Parameters. The blood distribution parameters, $K_{D,BC}$, and nP_T, were assumed to be equal in

Table II Physiological Parameters and Fraction of Tissue Vascular and Interstitial Spaces in Organs Used for the PBPK Model in a Human Standard Adult (70 kg) (18–20)

Organs	Total volume (l)	Blood flow (l/h)	Fraction of tissue vascular space	Fraction of interstitial space
Lung	1.17	262	0.262	0.188
Heart	0.27	9	0.262	0.100
Kidney	0.31	66	0.105	0.200
Bone	8.7	15	0.041	0.100
Muscle	30	45	0.026	0.120
Spleen	0.19	4.6	0.282	0.150
Liver	1.69	88.6	0.115	0.163
Intestines	1.65	66	0.024	0.094
Skin	7.8	18	0.019	0.302
Adipose tissue	10	15.6	0.010	0.135
Thymus	0.027	4.8	0.030	0.150
Venous blood	3.6	–	–	–
Arterial blood	1.8	–	–	–
Hepatic artery	–	18	–	–

adults and children. Cyclosporin fu_p varied as a function of age. Due to cyclosporin binding to lipoproteins, there is a strong correlation ($r^2=0.71$) between cholesterol concentration and cyclosporin fu_p: the higher the concentrations of cholesterol [CH] and triglyceride [TG], the lower the unbound fraction (30). This was expressed in the following equation (30):

$$fu_p = \frac{1}{1.346[TG] + 2.815[CH] + 1}$$

Values of cholesterol and triglyceride concentrations varied with age (Table III)(31,32). Thus, the unbound fraction of plasma cyclosporin in children was slightly different from that in adults: cyclosporin fu_p was 0.07 for patients under 15 years old and 0.06 for patients over 15.

For the cyclosporin tissue distribution, the ratio of lipoprotein concentrations, fu_T, and fu_I were assumed to be identical in children and adults. The adult values of k_{ass}, k_{dis}, and PS_{TC} were adjusted for children by multiplying by the ratio of body weights for children and adults. The B_T and $K_{D,TC}$ were assumed to be identical, per unit of organ mass, in children and adults (17).

Clearance. Hepatic intrinsic clearance in a standard adult ($CL_{int\text{-}adult}$) was set at 1470 l/h. Based on information on the ontogeny of specific CYPs (expression and activity per mg of microsomal proteins in the liver), the abundance of hepatic CYP 3A4/A5 in children was expressed according to age as a fraction of the abundance of CYP 3A4/A5 in adults (33). This fraction (f) was calculated as follows:

$$f = \frac{Age^{0.83}}{0.31 + Age^{0.83}}$$

Thus, the hepatic intrinsic clearance in a child, given the age, was calculated as $f \times CL_{int\text{-}adult}$. The evolution of blood clearance as a function of age (Fig. 2) was incorporated in the model. The range of values is consistent with the typical clearance of cyclosporin in pediatric patients (9 to 17 ml/min/kg, *i.e.*, 5 to 50 l/h for body weights of 10 to 50 kg (34)).

Fitting the PBPK Model in Children. The pediatric PBPK model was fitted individually to the venous whole blood cyclosporin concentrations of the 61 pediatric patients in the clinical study. For each patient, the data records included the dosing history, first two concentration measurements, and body weight, age, and hematocrit. The estimated parameters were the hepatic intrinsic clearance and the plasma unbound fraction of cyclosporin. A Bayesian estimator (Maximum *a Posteriori*) was used to estimate these parameters. The prior distributions were assumed log-normal and independent. Prior means were adjusted for each child according to age for CL_{int} (as $f \times CL_{int\text{-}adult}$) and to TG and CH for fu_p, respectively. The inter-individual coefficient of variation of both parameters was set at 30%. To account for the imprecision of cyclosporin concentration measurements, a proportionnal residual error model was used. The coefficient of variation of the residual error was assumed to be constant and was set to 15%. The areas under the concentration *versus* time curves (AUC from 0 to 24 h after the onset of treatment, and at day 13, because the median time to aGVHD was day 14) were estimated by numerical integration in several compartments (venous blood and interstitial compartments of bone, skin, liver, and intestines), using the dosing history of each child.

Table III Mean Concentrations of Triglycerides and Cholesterol According to Age of Children[31,32]

Age (y)	Mean triglycerides concentration (mmol/l)	Mean total cholesterol concentration (mmol/l)
5	0.86	4.40
10	0.86	4.27
15	1.03	3.62
20	1.03	4.91

Simulations in the Pediatric Population

Individual simulations of the PBPK model were carried out using several set of variables (body weight, age, hematocrit, dose, fu_p and CL_{int}) chosen according to the results of the clinical study. The AUCs were estimated in venous blood, in interstitial compartments of bone, skin, thymus, liver, kidney and intestines, and in intracellular compartment of kidney. The fractional receptor occupancy (RO) was calculated as

$$RO(t) = \frac{Cu(t)}{IC_{50} + Cu(t)}$$

where *Cu(t)* is the unbound concentration of cyclosporin, and IC_{50} its half-maximal inhibitory unbound concentration. The typical value of total IC_{50} for IL2 inhibition of production is 200 µg/l (depending on the measured effect, the estimates of total IC_{50} varies from 40 to 440 µg/l (35–37)), while the typical value of fu_B is 0.05. Therefore, the IC_{50} of unbound concentration of cyclosporin in blood was fixed at 10 µg/l. The area under the receptor occupancy *versus* time curves (AUC_{RO}; from 0 to 24 h after the onset of treatment) was calculated in venous blood, in interstitial compartments of skin, liver, kidney and intestines, and in the intracellular compartment of kidney.

To characterize the impact of nonlinearity in cyclosporin kinetics, the simulations of the nonlinear model described above were compared to simulations with the corresponding linear model. The linear model was obtained by multiplying $K_{D,BC}$, nP_T and the B_Ts, and Kd_{TC}s by 10,000 in the nonlinear model. The ratios of AUCs calculated by simulation of the nonlinear and linear models were used as index of nonlinearity.

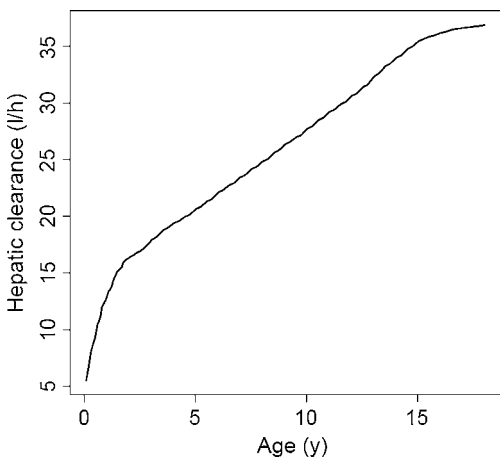

Fig. 2 Variation of cyclosporin hepatic clearance with age. [33]

Statistical Methods

Statistical analyses were performed with the SPSS package (version 17, SPSS, Chicago, Illinois, USA). A non-parametric test (Mann-Whitney test) was used to compare means, and the chi-square test was performed to compare proportions, with an alpha risk fixed at 5%. The goodness-of-fit of the model at the population level was assessed by visual examination of the predicted *versus* observed concentration plot, the proportion of predicted concentrations in the range between two-fold lower and two-fold greater than observed concentrations, and the histogram of weighted residuals. The latter distribution was compared to the normal distribution N (0,1) by the Shapiro-Wilk test. The bias of the fit was calculated as the mean of the prediction errors (PE), defined as the mean difference between the predicted and the observed concentration. The precision was calculated as the root of the mean squared prediction errors (RMSE).

RESULTS

Clinical Study

The two pediatric groups showed comparable demographics, HSCT indication, donor type, initial dosing regimen (Table IV), number of mismatches, and administration of anti-lymphocyte serum (data not shown). The cyclosporin dose range was nearly the same in both groups on day 1. At steady-state, the median dose was 67% higher in the II group than in the CI group. The clinical study showed that the frequency of aGVHD was similar in both groups: 60% after II, compared to 52% after CI. The mean grade was 0.74 after II, compared to 1.13 after CI.

Analysis of Cyclosporin Disposition

The simulated kinetic profiles of cyclosporin in the blood and all organs of the rat PBPK model were similar to the experimental data of Kawai (17) (figures not shown). Thus, the implementation of the rat PBPK model in the ADAPT II® platform was validated.

Table IV Patient Characteristics in Both Groups

n	Intermittent infusion 31	Continuous infusion 30	*p* value
Median age (range), y	7.58 (0.62–17.0)	5.73 (0.48–15.8)	0.942
Median body weight (range), kg	22.4 (5.62–63.0)	19.8 (7.18–58.6)	0.931
Sex (% male)	51.6	56.7	0.500
Hematocrit (range)	0.26 (0.14–0.32)	0.25 (0.16–0.31)	0.434
Disease			
Acute lymphoblastic leukemia (%)	25.8	26.7	0.50
Acute myeloid leukemia (%)	22.6	33.3	
Other malignant haematological diseases (%)	16.1	10	
Non malignant haematological diseases (%)	35.5	30	
Donor type			
Related (%)	61.3	43.3	0.20
Unrelated (%)	38.7	56.7	
Treatment			
Median dose at day 1 (range), mg/kg	3.14 (2.64–5.51)	3.12 (2.21–5.26)	0.243
Median dose at steady-state (range), mg/kg/d	3.92 (1.34–8.40)	2.35 (1.12–3.84)	<0.001
Median through concentration (II) or plateau concentration (CI) (range), μg/l	115 (41.0–248)	240 (79.0–490)	–

Goodness-of-Fit of the PBPK Model in Children

Plots of predicted *versus* observed concentrations in venous blood were analysed (Fig. 3). The bias and precision (RMSE) were −25.1 μg/l and 76.2 μg/l, respectively. More than 95% of the predicted concentrations were in the range between two-fold lower and two-fold greater than the observed concentrations. The weighted residual distribution was not significantly different from the normal distribution with zero mean and unit variance (Shapiro test W = 0.983, $p = 0.12$), and 100% of weighted residuals were in the range [−3;+3]. A plot showing observations and predictions over time for 8 children (4 with intermitent infusion and 4 with continuous infusion) is represented in Fig. 4.

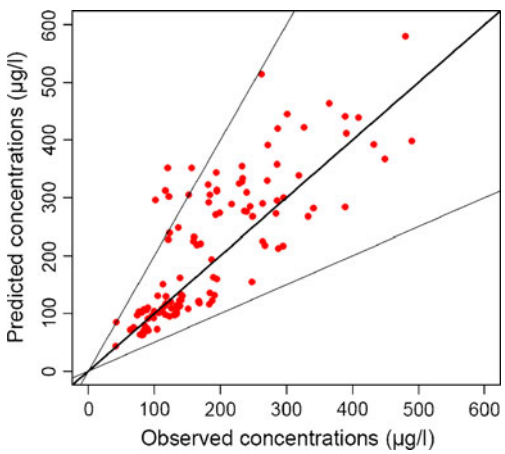

Fig. 3 Predicted *versus* observed concentrations of cyclosporin in venous blood. The thick line represents the identity line, and the thin lines are y = 2x and y = 0.5x.

Estimation of CL_{int} and fu_P

For CL_{int}, the median and range were 879 l/h (568–1381) and 1219 l/h (836–1463) for patients with intermitent infusion and patients with continuous infusion, respectively.

For fu_P, the median and range were 0.045 (0.029–0.066) and 0.063 l/h (0.040–0.075) for patients with intermitent infusion and patients with continuous infusion, respectively.

Comparisons of Exposure in Blood and Organs According to Infusion Type. With the fitted PBPK model, cyclosporin exposures, reflected by the AUCs in blood and aGVHD target organs, were calculated for each child. The results showed that at the beginning of treatment (0–24 h), the II showed higher cyclosporin exposure than the CI, with significantly greater AUCs in the blood, interstitial spaces of aGVHD target organs, and lymphoid organs (Fig. 5). However, these results might have been confounded, because this was a parallel group and not a crossover study. To further analyze the differences in cyclosporin exposure induced by both dosing schedules, a simulation study was undertaken.

Simulation Studies. The clinical study allowed us to define relevant sets of values for demographic variables, hematocrit, intrinsic clearance and fu_p for the simulation study (Table V).

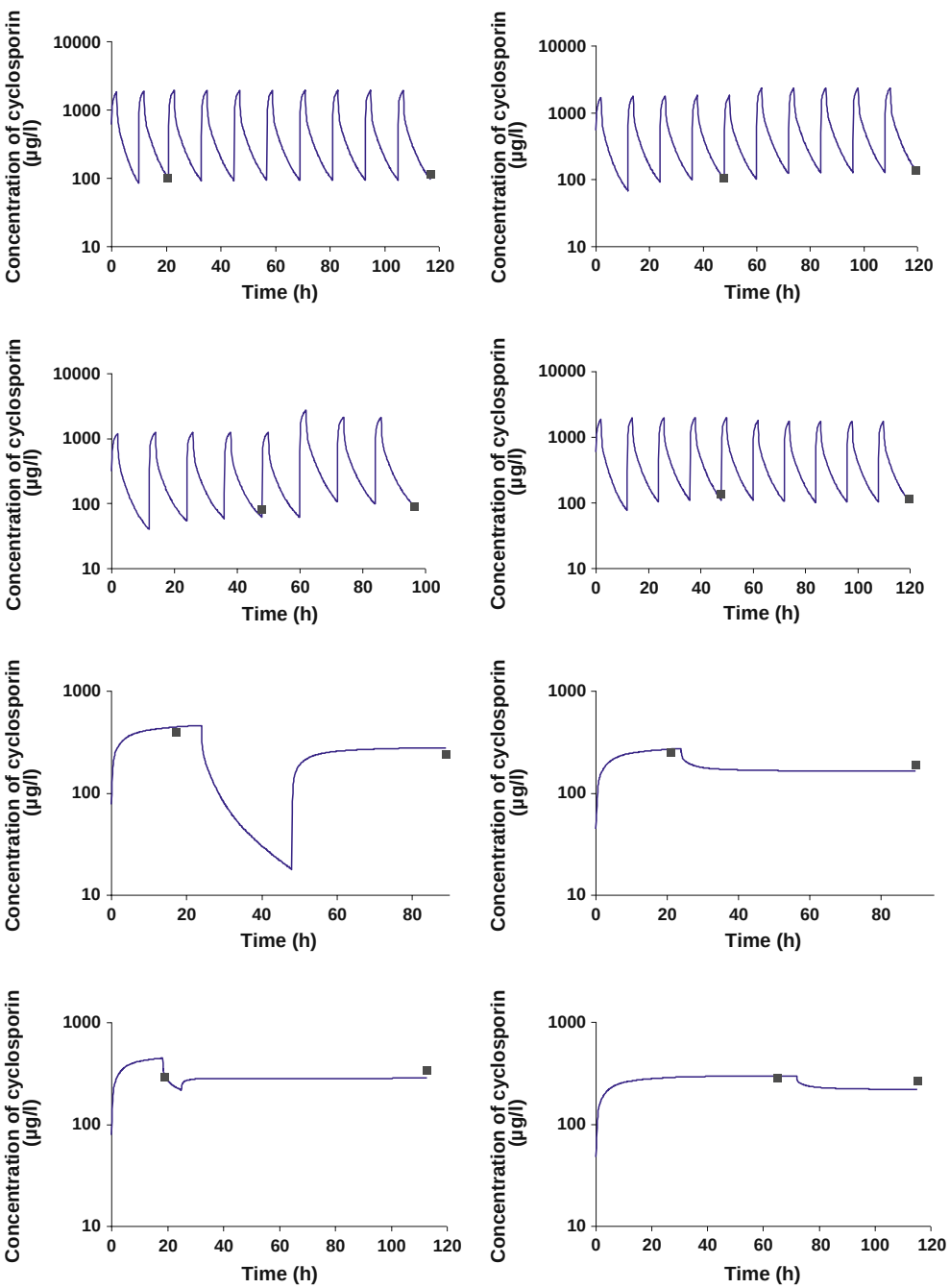

Fig. 4 Time course of cyclosporin concentration in blood for 4 patients with intermitent infusion and 4 patients with continuous infusion.

First, to compare AUCs in the GVHD target organs according to the type of infusion, an individual simulation was performed using the physiological parameters of a typical patient (age 6.5 y, body weight 26 kg, and hematocrit 0.26). The dosing regimen was 3 mg/kg/day, the intrinsic clearance was set at 1100 l/h (the median value in our population), and the unbound fraction of cyclosporin in plasma was set at 0.053. The change in interstitial cyclosporin concentration over time in the skin is shown in Fig. 6. During the first day of treatment, the exposure was greater after the II than after the CI. Similar curves were obtained in the other organs (data not shown). The simulation also showed that, upon repeated administration, the average concentration observed after the II or the CI was similar at day 2 and after.

Second, the exposures and receptor occupancies for the different simulation conditions of Table V were calculated. Table VI shows the results for the typical patient defined above. The variations of exposures and receptor occupancies resulting from the different simulation conditions were typically less than 10% from these values (data not shown). Regarding the impact of the dosing schedule (third column of Table VI), the interstitial AUCs calculated at day 1 were 7 to 13% greater after the II than after the CI, but the

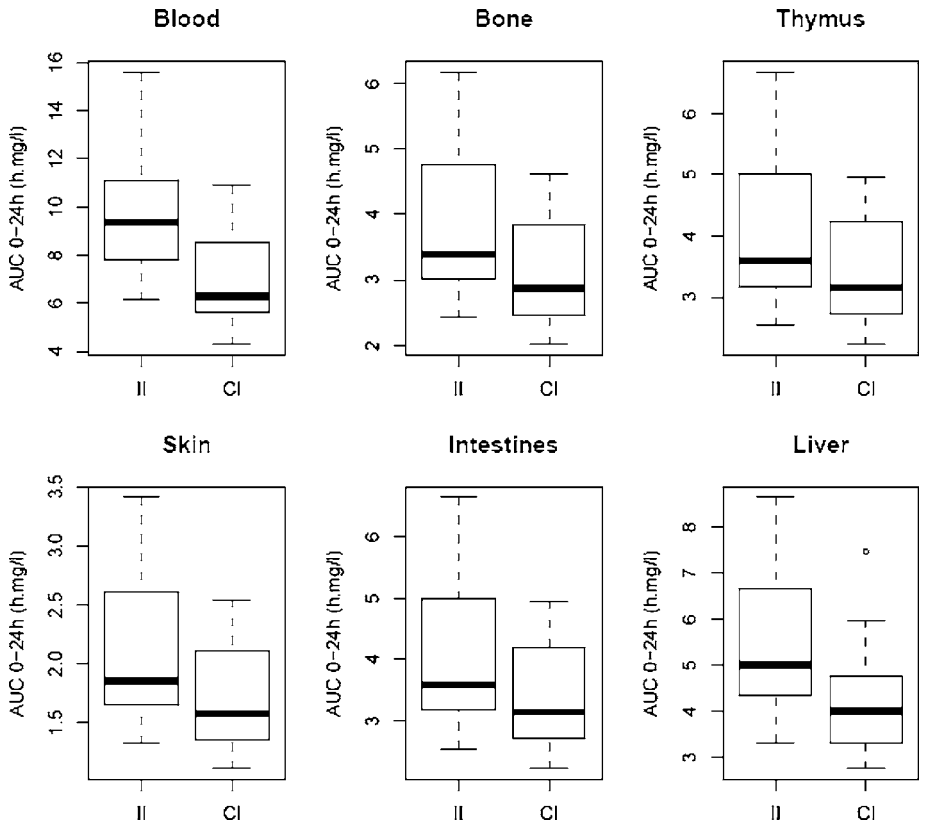

Fig. 5 Box plots of AUC 0–24 h estimated at day 1 in all children. AUCs are in the interstitial space, except for blood. All comparisons between II and CI are significant ($p < 0.05$).

corresponding AUC_{RO}s were 13 to 20% lower. This difference remained at a steady state. Finally, the total intracellular AUC in kidney was 13% lower after the II than after the CI. Regarding the influence of nonlinearity on cyclosporin disposition (column 4 and 5 of Table VI), the interstitial AUCs were 11 to 28% greater with the nonlinear model than with the linear model. Conversely, the total intracellular AUC in kidney estimated with the nonlinear model was, respectively, 66% and 53% lower after the II and the CI than with the linear model.

DISCUSSION

In this retrospective study, two carefully selected groups of patients were comparable in demographic characteristics and pathological status. The two groups differed in the mode of cyclosporin administration: one group received continuous and the other received intermittent infusions. Cyclosporin dose differed between both arms probably because the target concentrations were not equivalent, *i.e.* the target concentrations for II require higher doses to be

Table V Simulation Conditions

Body weight (kg)	Dose (mg/kg/d)	Hematocrit	Age (y)	fu_p	CL_{int} (L/h)
20	2	0.26	6.5	0.053	1100
20	3	0.26	6.5	0.053	1100
20	4	0.26	6.5	0.053	1100
20	3	0.26	6.5	0.053	800
20	3	0.26	6.5	0.053	500
20	3	0.16	6.5	0.053	1100
20	3	0.36	6.5	0.053	1100
20	3	0.26	6.5	0.033	1100
20	3	0.26	6.5	0.073	1100
10	3	0.26	1.5	0.053	1000
40	3	0.26	12.5	0.053	1400

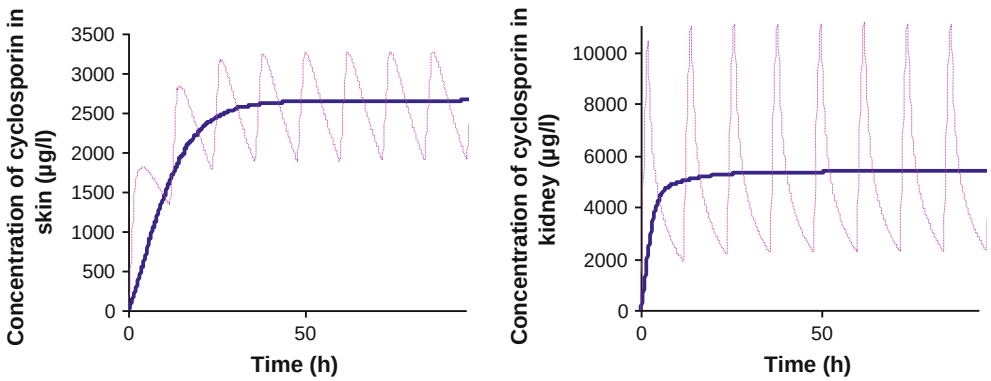

Fig. 6 Individual simulation of the time course of total cyclosporin in the skin (on the left) and in the kidney (on the right) with the two types of infusion (solid line for CI and dotted line for II).

reached than those for CI. Because cyclosporin dose and exposure were significantly different during treatment, no attempt was made to compare the efficacy in both arms. The PBPK model was used to simulate the kinetic profile of cyclosporin in the plasma, red blood cells, and interstitial and intracellular spaces of 11 organs. The model was also used to simulate the kinetic profile of receptor occupancy in blood and tissue cells. This simulation was possible because the unbound concentration of cyclosporin was modelled in all compartments, and the free IC_{50} for IL2 inhibition was approximately known. The effect on IL2 was considered because IL2 mediates T-lymphocyte activation, which plays a major role in GVHD. The model took into account diffusion-limited kinetics in most organs and the saturable binding of cyclosporin to red blood cells and to intracellular components. Thus, the model was *a priori* well suited for detecting differences in the pharmacokinetic profiles and receptor occupancies induced by different modes of drug administration. Saturation of binding to red blood cell may increase the proportion of cyclosporin in tissues, while saturation of binding to intracellular components may increase the proportion in blood and interstitial spaces, which contain the target cells for cyclosporin action. Saturation of binding depends, however, on the dose level and dosing schedule.

The simulation of the kinetic profiles for a typical child showed that the exposure in blood and interstitial space of organs was higher with the nonlinear model than with the linear model. The opposite was observed for the intracellular concentration in the kidney (and other tissues, data not shown). This difference shows that the saturation of intracellular binding of cyclosporin (not accounted for in

Table VI Exposures and Receptor Occupancies for the Typical Patient

Parameter		II-NL	CI-NL	NL-II/CI	II-NL/Lin	CI-NL/Lin	Lin-II/CI
AUC 24 h in interstitial space (h.mg/l)	blood	6.31	5.89	1.07	1.11	1.21	1.17
	skin	1.48	1.32	1.12	1.19	1.28	1.20
	intestines	2.88	2.61	1.10	1.19	1.28	1.19
	liver	3.60	3.26	1.10	1.18	1.28	1.19
	bone	2.72	2.40	1.13	1.20	1.28	1.21
	kidney	2.89	2.63	1.10	1.17	1.27	1.19
AUC_{RO} 24 h in interstitial space (h)	blood	10.3	12.9	0.80	0.89	1.11	1.00
	skin	10.8	12.4	0.87	0.94	1.15	1.07
	intestines	10.6	12.9	0.82	0.89	1.14	1.05
	liver	8.8	10.6	0.82	0.90	1.17	1.06
	kidney	10.4	12.9	0.81	0.88	1.14	1.04
AUC 24 h in intracellular space (h.mg/l)	kidney[a]	106.7	122.2	0.87	0.34	0.47	1.20
		116.6	135.9	0.86	0.29	0.33	1
AUC_{RO} 24 h in intracellular space (h)	kidney[a]	19.7	20.7	0.95	0.89	0.94	1.01
		20.2	21.1	0.96	0.89	0.92	1

CI continuous infusion, *II* intermittent infusion, *NL* stands for values calculated using the nonlinear model, *Lin* for linear model

[a] upper value : day 1; lower value: steady-state

the linear model) occurs at the usual dose, resulting in a lower proportion of cyclosporin in tissues and a higher proportion in the blood and interstitial fluid. This saturation, as reflected by the NL/Lin ratios of Table VI, is more pronounced with CI than with II in the interstitial space, while the opposite is observed in the intracellular space of tissues with non linear distribution.

On the other hand, the simulation of the kinetic profiles for a typical child with the nonlinear model showed that, given the same total cyclosporin dose, the exposure in blood and interstitial fluid of organs at day 1 was higher with II than with CI. This difference would also be observed in a linear system and would be more pronounced. In spite of higher exposure, receptor occupancy in blood and interstitial fluid of organs was about 20% lower with II than with CI. The latter result shows that II induces saturation of binding to its receptor at the usual dose level. The simulation also showed that, upon repeated administration, the average concentration in intersititial space of all organs observed after the II or the CI was similar at day 2. Hence, differences in the efficacy of these modes of administration could occur only in circumstances where 1) the exposure at day 1 played a major role or 2) the efficacy was linked to the whole profile of cyclosporin concentration, not the average concentration. In renal transplantation, the effect of exposure was significant in the early post transplantation period; during the 5 days post transplant, a lower risk of rejection was correlated with concentrations of cyclosporin greater than 1 500 μg/l at 2 h after administration (38,39). Furthermore, in hematopoietic stem cell transplantation, cyclosporin administration that started 7 days *versus* 1 day before transplantation, was associated with a trend of reduced probability of severe aGVHD (40) and a significantly higher disease-free survival (41). The CI exhibited higher receptor occupancy than II at day 1; this may result in an improved efficacy of CI.

However, tolerability must also be accounted for. The most frequently limiting side effect of cyclosporin is nephrotoxicity. The mechanism of nephrotoxicity involves altered transcription of genes in kidney cells, due to calcineurin inhibition (42). Hence, the very first step of cyclosporin-induced nephrotoxicity remains binding to intracellular cyclophilin. Simulations show that, given the same total cyclosporin dose, the exposure and the receptor occupancy in intracellular space of kidneys were higher with CI than with II, at day 1 and at steady state. This may result in a greater nephrotoxicity with CI than with II. However, the therapeutic index, roughly estimated as the ratio of AUC_{RO} in blood to AUC_{RO} in kidney cells, is 0.62 with CI *versus* 0.52 with II. The latter results suggest that CI may be slightly better than II.

CONCLUSION

The PBPK model of children showed that the exposure to cyclosporin in the interstitial fluid of aGVHD target organs was greater at day 1 after intermittent infusion than after continuous infusion, given at the same dose. The difference in interstitial exposure vanishes at day 2. By contrast, the exposure to cyclosporin in the intracellular space of kidney was greater at day 1 and at steady state after CI than after II. Finally, the model enabled the estimation of the therapeutic index associated with each mode of administration; it was slightly better after continuous infusion. Factors such as age, body weight, hematocrit, unbound fraction and intrinsic clearance had a moderate influence on the exposure to cyclosporin. Further analyses are required to determine whether cyclosporin efficacy in the prevention of aGVHD depends on the average concentration or on the entire concentration profile in the interstitial space of target organs (this is the topic of a companion paper). Ultimately, this information will contribute to determine the optimal mode of cyclosporin administration.

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