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RESEARCH PAPER

Links Between Cyclosporin Exposure in Tissues and Graft-*Versus*-Host Disease in Pediatric Bone Marrow Transplantation: Analysis by a PBPK Model

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ABSTRACT

Purpose In hematopoietic stem cell transplantation (HSCT), cyclosporin is used to prevent graft-*versus*-host disease (GVHD). However, cyclosporin distribution in tissues is not linear, resulting in uncertainty regarding optimal dosing and monitoring. The objective of this study was to link the probability and severity of acute GVHD to cyclosporin exposure in blood, GVHD target organs, and lymphoid organs.

Methods A physiologically based pharmacokinetic model of cyclosporin disposition and logistic regression models were used. Sixty-one pediatric patients undergoing HSCT were studied. Cyclosporin was administered by intermittent ($n = 31$) or continuous infusion ($n = 30$).

Results At steady state (1 day before acute GVHD), exposures in all organs were related with the probability and severity of acute GVHD. Average cyclosporin concentration or, equivalently, its area under the curve (AUC) was the pharmacokinetic index best correlated with the anti-GVHD effect. Cyclosporin AUC in interstitial fluid of lymphoid organs was a superior index than that in blood, but marginally.

Conclusion Hence, AUC in blood maybe used as an index of cyclosporin efficacy. Using our model, target AUCs in blood could be defined for malignant and non-malignant diseases, as well as the equivalent target values for C_2 and C_0 concentrations.

KEY WORDS bone marrow transplantation · cyclosporin · GVHD · PBPK modelling

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) may induce acute graft-*versus*-host disease (aGVHD), which mainly occurs in skin, liver, and intestines (1). Despite progress in prophylaxis, aGVHD is still responsible for significant morbidity and mortality in HSCT recipients (2). Cyclosporin, an immunosuppressant drug, is used to prevent aGVHD. This drug exhibits a relatively narrow therapeutic window (3) and large inter-individual pharmacokinetic variability. Therefore, in order to improve therapeutic efficacy and reduce aGVHD, it is critical to optimize the dose regimen of cyclosporin to achieve and maintain the concentration in the target range (4–6). Severe

GVHD carries a poor prognosis, with 25% long-term survival for grade III and 5% for grade IV (7).

In HSCT, cyclosporin is usually administered by intravenous infusion during the first 2 or 3 weeks following transplantation, before switching to the oral route. Continuous infusion (CI) and 2-h intermittent infusions (II) are commonly used, but there is no consensus regarding the best mode of administration (8). Our previous study, using a physiologically based pharmacokinetic model, showed that the area under the receptor occupancy *versus* time curve in the interstitial fluid of aGVHD target organs was greater after CI than after II, suggesting that CI maybe more efficacious (9).

There is also no consensus on the best way to monitor cyclosporin concentrations. The indices commonly considered for cyclosporin monitoring include trough concentration (C_0), 2-h concentration (C_2, in case of administration by 2-h infusion or by the oral route), plateau concentration (in case of continuous infusion), and AUC. In solid organ transplantation, concentration sampling 2 h after administration is usually considered as the best single method to predict exposure and effect (side effects and rate of rejection) (10–13). Following renal transplantation, both C_2 and C_0 were shown to be useful in predicting cyclosporin side effects (14). However, abbreviated AUC monitoring identified patients at risk for acute rejection more accurately than C_2 (15). In HSCT, C_0 was found to be a better predictor of aGVHD than C_2 (4,16). An inverse relationship also exists between the probability of severe aGVHD and the median cyclosporin concentration during the week before engraftment (17). Information regarding the possible association between aGVHD and cyclosporin AUC-12 h is not currently available (18).

The main objective of this study was to identify the pharmacokinetic index of cyclosporin exposure best correlated with aGVHD occurrence and severity. The second objective was to determine the target values of this index to maximize the probability of obtaining the desired grade of GVHD in malignant and non-malignant diseases.

MATERIALS AND METHODS

Clinical Study

This was a retrospective, monocentric study. We studied the records of pediatric patients (4 months to 17 years of age) that underwent allogeneic bone marrow transplantation and received cyclosporin for the prevention of graft-*versus*-host disease (GVHD). The patients gave a written consent to use their data at the time of admission. Cyclosporin treatment was 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 for age, sex, indication for transplantation (malignant *vs.* 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, Deerfield, Illinois, USA) on a Cobas Mira® analyzer automate. 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 (4,19). 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 aGVHD was graded according to the classification of Glucksberg modified by Armitage (20–22). This scale determines aGVHD severity (from grade 0 to IV, no aGVHD to maximum severity) by a combination of the scores of the three target organs (skin, intestines and liver) of aGVHD. These organs are graded independently from 0 to 4, corresponding to the extent of skin rash, diarrhea volume, and total bilirubin concentrations.

PBPK Model in Pediatric Patients and Predicted Exposure

The pediatric physiologically based pharmacokinetic (PBPK) model, including 11 organs validated in children (9), was fitted individually to the venous whole-blood cyclosporin concentrations of the 61 pediatric patients undergoing HSCT and receiving only cyclosporin as immunosuppressive treatment. For each patient, the data records included the dosing history, the first two concentration measurements, 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, as described in our previous study (9). The areas under the concentration *vs.* time curves (AUC D1, from 0 to 24 h after the onset of treatment, and AUC SS, at steady state) were estimated by numerical integration in several compartments (venous blood and interstitial compartments of bone including bone marrow, skin, liver, thymus, and intestines), using the dosing history

of each child. The time corresponding to steady state was defined as the day before the occurrence of aGVHD, or day 13 when no aGVHD occurred (median time to aGVHD was 14 days). A three-compartment open model was fitted to the individual concentration profiles in blood, simulated thanks to the PBPK model. The kinetic profiles obtained with the three-compartment model were indistinguishable from the profiles generated by the PBPK model. The three-compartment model was coupled with the interface model (described below) and allowed to calculate $C_b(t)$. Substituting the three-compartment model to the PBPK model was necessary for computational reasons, because the software used for subsequent analyses (NONMEM) could not easily handle a PBPK model involving a large system of ordinary differential equations.

Interface Model

In order to test the influence of the concentration profile (II *versus* CI) on the efficacy of cyclosporin (in terms of aGVHD), an interface model was constructed. This approach was introduced recently (23) for relating the drug concentration profile in the body and the pharmacodynamic (PD) response. This model is a nonlinear-effect compartment model that ensures the required sensitivity of the PD response with respect to drug dose and/or concentration variation. The concentration in the effect compartment, C_e, varies according to the following equation:

$$dC_e/dt = -\alpha.C_e.\exp(-\beta.C_e) + H.(C_b - C_{th})$$

where C_b is the blood cyclosporin concentration calculated using the three-compartment model, C_{th} is the threshold concentration, and H is a categorical variable equal to 1 if C_b is greater than C_{th}, and equal to zero otherwise. The parameter α is the elimination rate constant from the effect compartment; it controls the delay between the kinetics of concentration in blood and the kinetics of effect. The parameter β controls the saturation mechanism from the effect compartment. When $C_{th}=0$ and $\beta=0$, this model reduces to the usual effect compartment model. A nonlinearity in the transduction process would result in C_{th} or β greater than 0. The average concentration in the effect compartment over the preceding 24 h, C_{av}, was calculated as the 0–24 h AUC of $Ce(t)$, divided by 24. It was taken as a pharmacokinetic index to be related to the probability P of occurrence of aGVHD in a binary logistic regression model:

$$Logit(P) = a_0 - a_1.C_{av}$$

where a_0 and a_1 are regression parameters to be estimated. The parameter a_0 controls the probability of aGVHD when no drug is given, while a_1 controls the rate of reduction of this probability by cyclosporin concentration unit. Because the estimates of parameters α and a_1 were expected to be highly correlated, the parameter α was fixed to 1 so that at equilibrium, under the null hypothesis $C_{th}=0$ and $\beta=0$, the concentrations C_e and C_b are equal. Hence, the parameters a_0, a_1, β, and C_{th} were estimated by nonlinear regression, while the remaining parameters were fixed either to a common value ($\alpha=1$) or to their individual estimate (pharmacokinetic parameters). The likelihood ratio test was used to test the hypothesis $C_{th}=0$ and $\beta=0$.

Binary Analysis of aGVHD Occurrence

In this analysis, patients were quoted as 0 (no aGVHD) when their Glucksberg's score was equal to 0, and quoted as 1 otherwise. First, the mean predicted AUC D1 and AUC SS in blood, and interstitial fluid of bone, thymus, and intestines were compared according to the occurrence of aGVHD. In the same way, AUC D1 and AUC SS in blood and skin were compared according to the occurrence of cutaneous aGVHD. Second, the influence of cyclosporin exposure and other covariates on the probabilities of aGVHD and aGVHD in skin was assessed by binary logistic regression with SPSS package (version 17, SPSS, Chicago, Illinois, USA). The covariates considered were AUC, age, body weight, sex, and infusion type. The logit probability of aGVHD was modeled as a linear function of these covariates. Significance of the relationship was assessed by comparing the slope of the linear function to zero by a *t*-test.

Categorical Analysis of aGVHD Occurrence

In this analysis, the five-grade aGVHD classification of Glucksberg (20–22) was used. In order to visualize the relationship between exposure and aGVHD grades, an exploratory graphical analysis was first performed by plotting the cumulative probabilities of Glucksberg's grades as a function of cyclosporin AUC tertiles. Second, the mean AUCs in blood and organs by aGVHD grade (grades 0, 1, and ≥ 2) were compared by analysis of variance (ANOVA). Third, the links between cyclosporin exposure and total aGVHD grade or cutaneous aGVHD score were assessed by multinomial logistic regression. The logit of the cumulated probability of having a grade lower than a given value was modeled as a linear or nonlinear (E_{max}) function of exposure.

The linear model was defined by the following equation:

$$Logit[P(Y \leq m)] = A_m + B.AUC$$

where A_m and B were regression parameters to be estimated. The E_{max} model was defined by the following equation:

$$Logit[P(Y \leq m)] = A_m + \frac{E_{max}.AUC^{\gamma}}{AUC_{50}^{\gamma} + AUC^{\gamma}}$$

where E_{max} and AUC_{50} represent the maximum effect and the AUC producing 50% of the maximum effect, respectively. γ is the coefficient of sigmoidicity.

No random effect was considered. Parameter estimation was performed using the Laplacian method for likelihood computation in NONMEM (version VI, NONMEM Project Group, University of California, San Francisco, CA). In order to test the model's ability to predict the probability of observing a grade, qualification of the PD model was based on a visual predictive check. The distribution of predicted probabilities of aGVHD grades (0, 1, or ≥ 2) as a function of AUC SS tertiles in blood and intestines, calculated according to the logistic multinomial model, were compared with the observed probabilities. The distribution of the predicted probabilities was obtained by Monte-Carlo simulation, using the posterior distribution of the regression parameters of the multinomial model. The posterior distribution was assumed normal, with mean equal to the point estimate, and standard deviation equal to the standard error of the estimate.

Statistical and Pharmacokinetic Methods

Statistical analyses, including binary logistic regression, were performed using the SPSS package. Student's *t*-test was used to compare means, and the chi-square test was performed to compare proportions, with an alpha risk fixed at 5%. Pharmacokinetic and other non-linear regression analyses were carried out using the program NONMEM. For hypothesis testing during model building, the likelihood ratio test was used in case of nested models, based on the difference between the objective function values of the full and the reduced model, with alpha=5%. The Akaike's Information Criterion (AIC) was also calculated for model selection.

RESULTS

Clinical Study

Patient characteristics are summarized in Table I. An episode of aGVHD occurred in 34 patients (56%). In most cases, the disease was limited to skin; digestive and hepatic disease occurred only in approximately 10% of the patients. Therefore, only the grade of GVHD and the score of skin GVHD were considered in the analysis. The mean dose at day 1 was not significantly different in patients, whether aGVHD occurred or not (3.40 ± 0.80 *vs.* 3.93 ± 1.01 mg/kg, p-value$=0.10$).

Table I Patient Characteristics

n	61
Median age (range), y	6.72 (0.5–17)
Median body weight (range), kg	21.1 (5.6–63)
Sex (% male)	54.5
Disease (%)	
Acute lymphoblastic leukemia	26.2
Acute myeloid leukemia	27.9
Other malignant hematological diseases	13.1
Non–malignant hematological diseases	31.2
Metabolic diseases	1.6
Donor type (%)	
Related	52.5
Unrelated	47.5
aGVHD (n)	
Total aGVHD	34
Cutaneous aGVHD	32
Digestive aGVHD	7
Hepatic aGVHD	6
Infusion type (n)	
Intermittent	31
Continuous	30

Interface Model

In the context of the interface model, the average concentration of cyclosporin in the effect compartment was significantly related to the probability of aGVHD occurrence, when C_{th} and β were set to zero ($p<0.001$ compared to a model with $a_1=0$). Relaxing each assumption ($C_{th}>0$ or $\beta>0$) did not improve the fit; the difference in objective function values was less than 1. Hence, there was no evidence of nonlinearity in the effect compartment (at least those types of nonlinearities), and the transduction (in a broad sense, the link between receptor occupancy and anti-GVHD effect) could be regarded as a linear process. The probability of aGVHD was related to the average concentration, or equivalently, to the AUC over 24 h. Therefore, cyclosporin AUC was retained as a pharmacokinetic index in the pharmacodynamic analysis.

Cyclosporin AUC and Probability of aGVHD

The PBPK model, fitted to the individual data of each child, allowed the estimation of cyclosporin AUCs in blood and in the interstitial fluid of the target organs. At day 1, mean AUCs were significantly different between patients

Table II Comparison of Mean AUC 0–24 h and AUC SS as a Function of Outcome

		aGVHD (n = 34)	*No aGVHD (n = 27)*	*p*-value, *t*-test
Mean AUC D1 ± SD (h.mg/l)	Blood	8.16 ± 2.54	8.76 ± 2.70	0.380
	Bone	3.22 ± 0.83	3.79 ± 1.11	0.032
	Thymus/Intestines	3.46 ± 0.88	4.05 ± 1.14	0.031
Mean AUC SS ± SD (h.mg/l)	Blood	7.52 ± 3.06	10.1 ± 4.61	0.011
	Bone	3.26 ± 1.34	4.67 ± 2.06	0.004
	Thymus/Intestines	3.27 ± 1.36	4.69 ± 2.07	0.004
		Cutaneous aGVHD (n = 32)	*No cutaneous aGVHD (n = 29)*	
Mean AUC D1 ± SD (h.mg/l)	Skin	1.75 ± 0.45	2.08 ± 0.60	0.02
	Blood	8.14 ± 2.55	8.74 ± 2.69	0.38
Mean AUC SS ± SD (h.mg/l)	Skin	1.81 ± 0.76	2.50 ± 1.13	0.007
	Blood	7.63 ± 3.13	9.82 ± 4.58	0.035

with and without aGVHD, in bone and lymphoid organs (p-value < 0.05), but not in blood. At steady state, mean AUC in the same tissues was significantly greater when there was no aGVHD ($p < 0.05$ in each case, Table II). Regarding cutaneous aGVHD, at day 1 and at steady state, the mean AUC in skin was significantly lower for patients who suffered cutaneous aGVHD, compared to those with no cutaneous aGVHD (p-value < 0.05). In contrast, no significant difference was found for the mean AUC in blood between these two groups at day 1 (Table II).

These results prompted us to characterize quantitatively the link between the probability of GVHD and cyclosporin exposure. At day 1, no link between blood cyclosporin AUC and the occurrence of aGVHD was found by binary logistic regression (Table III). Conversely, there were significant links between interstitial AUCs in lymphoid organs at the beginning of the treatment (AUC D1) and the occurrence of aGVHD (p-value < 0.05). In the same way, AUC in skin was significantly related to the probability of cutaneous aGVHD (p-value < 0.05). At steady state, there were significant links between AUCs in blood and lymphoid organs and the occurrence of aGVHD ($p < 0.01$ in each case, Table III).

Sex, body weight, type of donor (related or unrelated), number of cells in the graft, and type of infusion were also tested as covariates. However, no covariate was found to be related to the outcome (data not shown). Cyclosporin infusion duration was not a significant covariate of aGVHD occurrence once AUCs were taken into account. When binary logistic regression took into account both AUC SS and AUC D1, only AUC SS remained a significant covariate (p-value < 0.05, data not shown). A 3D representation of the probability of aGVHD as a function of AUC D1 and AUC SS in intestines, the most influential organ, is shown in Fig. 1. The figure shows that AUC SS variations have a greater influence on aGVHD probability than AUC D1.

Table III Binary Logistic Regression. Logit(probability of aGvHD) = $a_0 + a_1$*AUC, p-value for $a_1 = 0$

		Probability	a_1	a_0	*p*-value, *t*-test
AUC D1	Blood		−0.09	0.991	0.37
	Bone	aGVHD	−0.611	2.36	0.03
	Thymus/ Intestines		−0.588	2.43	0.03
	Skin	Cutaneous aGVHD	−1.19	2.37	0.04
AUC SS	Blood		−0.177	1.77	0.016
	Bone	aGVHD	−0.506	2.19	0.006
	Thymus/ Intestines		−0.503	2.18	0.006
	Skin	Cutaneous aGVHD	−0.812	1.82	0.012

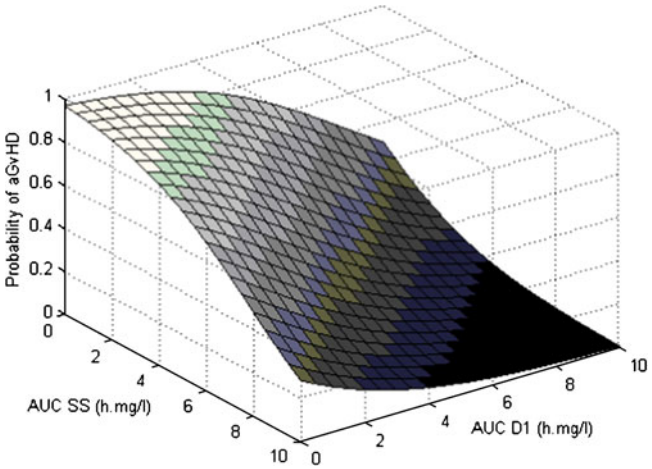

Fig. 1 3D representation of the probability of aGVHD function of AUC D1 and AUC SS in intestines. Logit(probability of aGVHD) = 3.36 − 0.39*AUC D1 − 0.43*AUC SS.

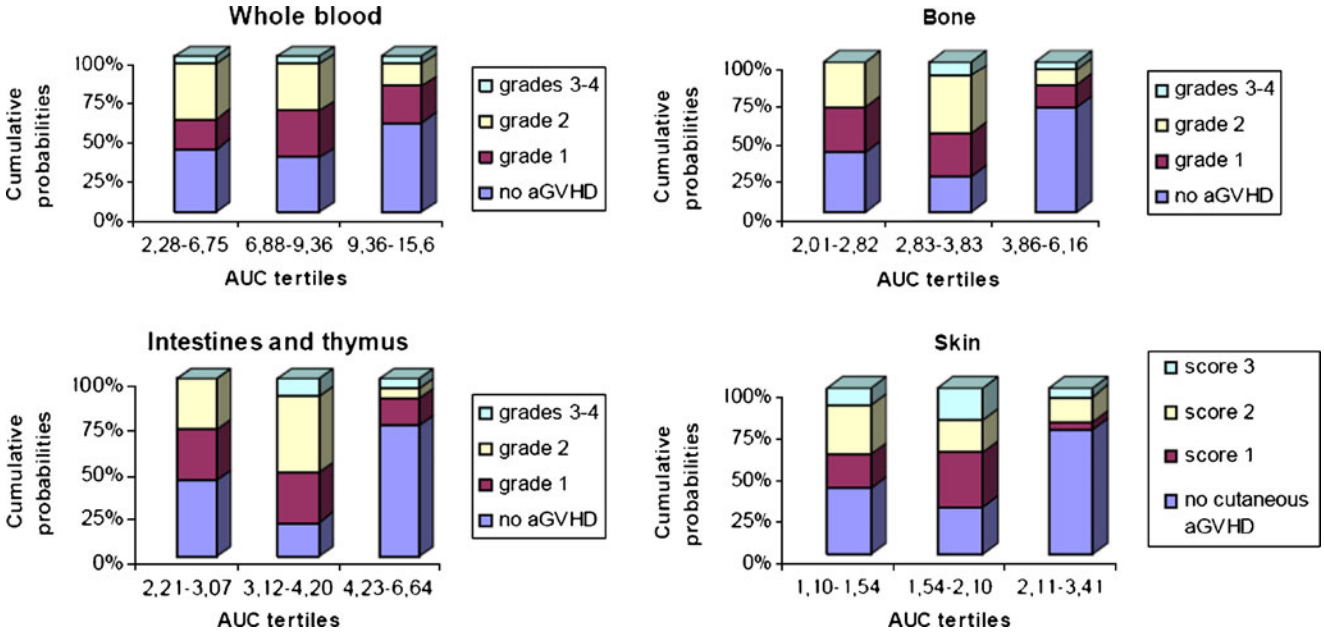

Fig. 2 Cumulative probabilities of GVHD grades as a function of AUC at day 1 in blood, bone, thymus, intestines, and skin.

Cyclosporin AUC and aGVHD Grade

Graphical exploratory analysis revealed that total aGVHD grades and cutaneous aGVHD scores were apparently not related to cyclosporin exposure at day 1. On the contrary, at steady state, high cyclosporin exposure in blood and interstitial fluids seemed to be associated with higher probabilities of low-grade GVHD (Figs. 2 and 3). At the beginning of cyclosporin treatment, for all lymphoid organs but not for the blood, aGVHD grade increased when there was a lower mean AUC D1. At steady state, for blood and lymphoid organs, the same trend was observed, and the difference between the three grades of aGVHD was statistically significant ($p<0.01$, Table IV).

Regarding total aGVHD, AUC SS was a more significant covariate than AUC D1 (p-value<0.01). The

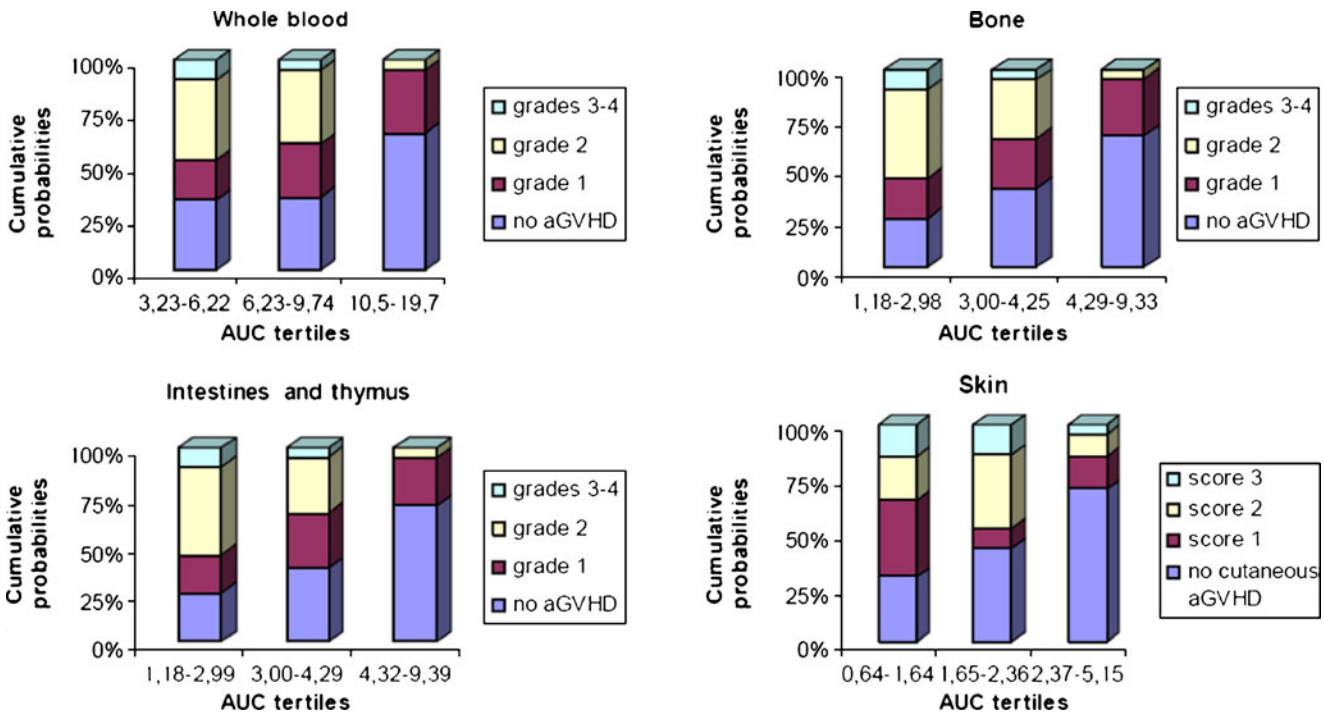

Fig. 3 Cumulative probabilities of GVHD grades as a function of AUC at steady state in blood, bone, thymus, intestines, and skin.

Table IV Mean AUC as a Function of Outcome (Grade of aGVHD)

		aGVHD			
		Grade 0 ($n=27$)	Grade 1 ($n=15$)	Grade ≥ 2 ($n=19$)	p-value, t-test
Mean AUC 0–24 h ± SD (h.mg/l)	Blood	8.76 ± 2.70	8.87 ± 3.27	7.60 ± 1.66	0.250
	Bone	3.79 ± 1.11	3.33 ± 1.02	3.14 ± 0.67	0.072
	Thymus/Intestines	4.05 ± 1.14	3.55 ± 1.07	3.39 ± 0.72	0.076
Mean AUC SS ± SD (h.mg/l)	Blood	10.1 ± 4.61	8.74 ± 3.02	6.56 ± 2.81	0.010
	Bone	4.67 ± 2.06	3.61 ± 1.22	2.99 ± 1.42	0.005
	Thymus/Intestines	4.69 ± 2.07	3.62 ± 1.22	3.00 ± 1.43	0.005

E_{max} model was not significantly better than the linear model. Grade of aGVHD was significantly related to AUC SS in blood and in bone, intestines, and thymus ($p<0.01$). According to the criterion of Akaike, the best model was the linear model involving AUC SS in organs, although the difference with the model involving AUC SS in blood was almost as good (AIC = 126.4 *vs.* 124.2). No other potential covariate exerted a significant influence (data not shown). The parameter values of the best model are summarized in Table V. The predictive check revealed no invalidation of the model (Fig. 4). The probability of aGVHD grades (0, 1, or ≥ 2) as a function of AUC SS in blood, according to the logistic multinomial model, is shown in Fig. 5.

DISCUSSION

The main objective of this statistical analysis, based on a modeling approach, was to identify the pharmacokinetic index of cyclosporin exposure best correlated with aGVHD severity. The indices commonly considered for cyclosporin include the trough concentration, 2-h concentration, plateau concentration, and AUC. For practical reasons, all these indices are measured in blood. In our study, patients received cyclosporin by CI or II. In this way, a similar average concentration or AUC could be achieved through very different concentration profiles (with different C_2 and C_{12} concentrations). This design, coupled with the analysis by an interface model, allowed us to disentangle the influence of the concentration profile in blood, the hypothetical delay due to cyclosporin diffusion to the receptor, and hypothetical nonlinearity in the transduction process.

We found that average concentration was the pharmacokinetic index related to the anti-GVHD effect, irrespective of the concentration profile. As a consequence, administration of cyclosporin by II or CI may have equal efficacies, provided that the average blood concentration achieved is similar. The higher AUC of receptor occupancy in the interstitial fluid of aGVHD target organs yielded by CI, compared to II in our previous study (9), does not result in increased efficacy, probably because the difference in receptor occupancy is too small. As AUC is equal to the product of average concentration and dosing interval, AUC 0–24 h may also be used as a predictor of efficacy.

AUC D1 and AUC SS were both related to anti-GVHD efficacy in univariate analysis, but AUC D1 was no longer a predictor once AUC SS was taken into account in a multivariate analysis. This result may be explained by the high correlation between AUC D1 and AUC SS; it does not imply that the early achievement of adequate AUC is not important for prevention of aGVHD.

Cyclosporin AUC in interstitial fluid of lymphoid organs was a more significant index than that in blood, but only marginally, because AUCs in blood and interstitial fluids were correlated (data not shown). As a consequence, considering interstitial AUCs is not a beneficial strategy, and target AUCs in blood may be defined. Two cases should be considered.

Table V Parameter Values of the Best[a] Model for aGVHD Grade (RSE: Relative Standard Error)

Total aGVHD linear model with AUC SS in intestines	Estimate	RSE (%)
Logit of baseline probability, grade < 1 (baseline probability, %)	−2.4 (8)	36
Logit of baseline probability, grade < 2 (baseline probability, %)	−1.2 (23)	24
slope B ($l.mg^{-1}.h^{-1}$)	0.56	37
Total aGVHD linear model with AUC SS in blood	Estimate	RSE (%)
Logit of baseline probability, grade < 1 (baseline probability, %)	−2.13 (11)	36
Logit of baseline probability, grade < 2 (baseline probability, %)	−0.96 (28)	24
slope B ($l.mg^{-1}.h^{-1}$)	0.214	35

[a] the best model is the model with the lowest AIC value

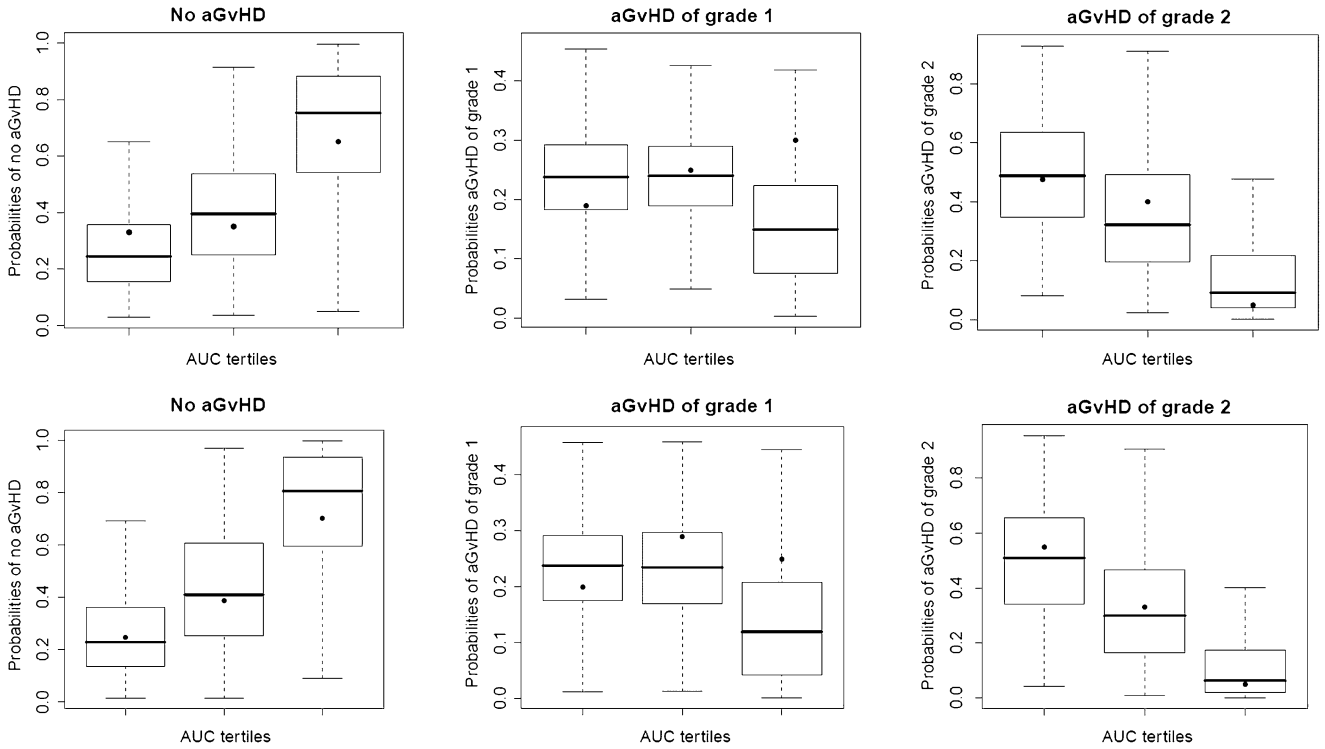

Fig. 4 Probability of aGVHD grades (0, 1 or ≥ 2) as a function of AUC SS tertiles in blood (*upper panel*) and intestine (*lower panel*), according to the logistic multinomial model. Each *box-plot* represent the distribution of the predicted probability, obtained by Monte-carlo simulation of the posterior distribution of the regression parameters of the model. The observed probability is represented by the *full circle* (●).

In the case of malignant disease, a grade 1 GVHD is desired for its anti-leukemia effect (24). A target AUC of approximately 10 h.mg/l may be chosen in order to maximize the probability of grade 1, but minimize the probability of grade ≥ 2 (Fig. 5). This target AUC is equivalent to an average cyclosporin concentration of 420 µg/l. According to our pharmacokinetic model, under 2-h intermittent infusion, the corresponding values for C_2 and C_{12} are 1,000 µg/l and 80 µg/l, respectively. These predictions agree with the target concentrations used in several studies: plateau concentration of 450 to 520 µg/l (25), 150 to 400 µg/l (26), 250 to 400 µg/l (27), C_2 observed by Hendriks (28) or Barkholt (16) and C_{12} observed by Martin (4). The agreement of our prediction with target values considered as relevant on clinical grounds is strongly supportive of our model. As a result, the model may be used to explore *in silico*, by simulation, the efficacy of different dosing strategies.

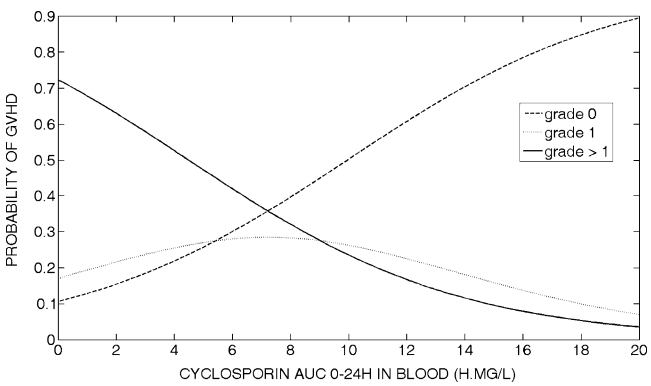

Fig. 5 Probabilty of aGVHD grades (0, 1, or ≥ 2) as a function of AUC SS in blood, according to the logistic multinomial model.

On the other hand, in the case of non-malignant disease, a grade 0 GVHD is desired. The target exposure is therefore different. An AUC of approximately 16 h.mg/l is associated with the absence of GVHD with a probability of 80% (Fig. 5). This value is equivalent to a plateau concentration of 660 µg/l, a C_2 of 1,600 µg/l, and a C_{12} of 120 µg/l.

CONCLUSION

In HSCT, the best pharmacokinetic index relating the anti-GVHD effect to cyclosporin exposure was its average concentration or, equivalently, its AUC. This finding is an indirect argument for equal efficacies of CI and II. Cyclosporin AUC in interstitial fluid of lymphoid organs was a better index than in blood, but only marginally. Because AUCs are much more easily measured in blood than in interstitial fluids, AUC in blood may be used as an index of cyclosporin efficacy. We employed a logistic model and a PBPK model to define target AUCs in blood, as well

as the equivalent target values for C_2 and C_{12} concentrations. These results may help to better design clinical studies aimed at comparing dosing strategies, rates of infusion and/or targets for therapeutic drug monitoring.

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