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Higher Therapeutic Cyclosporine Levels Early Post-Transplantation Reduces Risks of Acute Graft-Versus-Host Disease and Improves Survival

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Abstract

We studied whether early cyclosporine A (CsA) trough levels were associated with the risk of acute graft-vs.-host disease (GVHD) in 337 patients after either sibling peripheral blood stem cell or double umbilical cord blood transplantation. All patients, regardless of donor type, started CsA at a dose of 5 mg/kg IV divided twice daily, targeting trough concentrations 200–400 ng/ml. The CsA level was studied by a weighted average method calculated by giving 70% of the weight to the level that was measured just prior to the onset of the event or day +30. We found that higher weighted average CsA trough levels early post-transplantation contributed to lower risk of acute GVHD, and lower non-relapse and overall mortality. Thus, our data support close monitoring with active adjustments of CsA dosing to maintain therapeutic CsA levels in the first weeks of allo-HCT. In patients who are near or even modestly above the CsA target trough level, in the absence of CsA related toxicity, dose reduction should be cautious in order to avoid subtherapeutic drug levels resulting in higher risks for acute GVHD.

Keywords

Allogeneic transplantation; calcineurin inhibitor; cyclosporine; graft-vs.-host disease

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is a treatment option for many malignant and non-malignant disease states. Acute graft versus host disease (GVHD) occurs frequently following allo-HCT as a result of alloreactivity of immunocompentent graft cells against host antigens (1). GVHD, both in its acute and chronic forms continues to be a major source of morbidity and mortality following allo-HCT. Age, donor-recipient gender, CMV serostatus, ABO compatibility, disease, disease status, transplant source, donor type, HLA

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CONFLICT OF INTEREST

The authors have no relevant conflict of interests to disclose.

matching between donor and recipient, conditioning regimen intensity and GVHD prophylaxis have been shown to be risk factors for developing aGVHD. Cyclosporine (CsA), a calcineurin inhibitor, is one of the most commonly used pharmacologic agents for the prevention of GVHD following alloHCT, however, the dose, target blood level and schedule of administration varies among institutions. The association between CsA therapeutic blood levels post-transplantation and the development of acute GVHD has been documented in some but not all settings and regimens of administration (2–6). Thus, we retrospectively studied whether early CsA levels were associated with the incidence of acute GVHD in patients undergoing allo-HCT from either HLA-matched sibling (SIB) peripheral blood or unrelated double umbilical cord blood (dUCB) grafts.

PATIENT AND METHODS

Our study included patients ≥ 15 years, who were undergoing their first allo-HCT for a hematologic malignancy, between 2006 and 2010 at the University of Minnesota Medical Center. They received peripheral blood stem cells from a SIB or a dUCB graft. All SIB donors were 6/6 allele level HLA-matched to the recipient. The dUCB grafts were 4–6/6 HLA-matched to the recipient and to each other, considering HLA A and B at the antigen level and DRB1 at the allele level, as reported (7). Patients received either myeloablative or nonmyeloablative conditioning regimens, as described (7–10). All patients received CsA starting intravenously (IV) on day −3 at a dose of 5 mg/kg IV divided twice daily, targeting trough concentrations 200–400 ng/ml on day −1. Trough levels <200 ng/ml on day −1 were adjusted by increasing the CsA dose by at least 25%. CsA levels were monitored thrice weekly through day +7 then once weekly or 48 hours after a dose adjustment was made. Twelve hour trough whole blood samples were collected and measured by HPLC (11). Recipients of nonmyeloablative, regardless of donor type, and myeloablative conditioning with a dUCB graft also received mycophenolate mofetil (MMF) 2–3 g/day starting on day −3 until day +30, as reported (7, 8, 10). Recipients of myeloablative sibling transplants received methotrexate 15 mg/m2 on day +1 and 10 mg/m2 on days +3, +6 and +11 (9). Supportive care followed institutional guidelines and was the same regardless of donor type or intensity of the conditioning regimen as reported (7, 10)

Data Collection and Definitions

Demographic, graft and transplant characteristics as well as clinical outcomes were collected prospectively by the University of Minnesota Blood and Marrow Transplant Program Database. The CsA levels were collected retrospectively by chart review. All CsA levels available within the first 30 days of transplantation were recorded. Acute GVHD was staged using established criteria (12). Given that there were possible interactions between conditioning regimen, immunosuppression regimens and donor type patients, we divided the patients in four groups: 1) Myeloablative SIB, 2) Non-myeloablative SIB, 3) Myeloablative dUCB and 4) Non-myeloablative dUCB.

Statistical Analysis

Comparison of factors by donor type and conditioning were evaluated by the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. The

primary factor of CsA level post-HSCT was measured for every individual from day −1 through day +30. In assessing the effect of CsA on the endpoints of grade II–IV acute GVHD, grade III–IV acute GVHD, chronic GVHD, non-relapse mortality and overall survival, Cox regression was implemented using CsA as a time-dependent weighted average from day −1 to the measurement just prior to the event (e.g. GVHD, mortality) or the end of the observation period on day +30, whichever came first (13). Cox regression was used versus a competing risk model to accommodate the use of the time-dependent average level of CsA. Only 8 (2%) patients died prior to day 30 and thus the effect of informative censoring by early death was potentially minimal. The weighted average of CsA was calculated by giving 70% of the weight to the CsA level that was measured just prior to the event of interest or the end of the 30 day period post-transplantation. While the 70% weight was recommended by the authors of this particular method which was ultimately used (14), weights of 40%, 50%, 60% and 80% were tested in the models. The analysis up to day +30 using time-dependent weighted average described above did not use any measurements post onset of GVHD. The weighted average of CsA was analyzed by assessing the effect of a 50 ng/mL increase on the endpoints (14). Other factors considered as potential confounders in the regression analyses were ATG (yes versus no), age at transplant (15–34 versus 35–54 versus 55+), patient CMV serostatus (negative versus positive), gender (male versus female), disease risk (standard versus high) and donor type and conditioning intensity (Myeloablative SIB versus non-myeloablative SIB versus myeloablative dUCB versus non-myeloablative dUCB). Disease risk at the time of HCT was classified into standard risk or high risk based on the American Society of Blood and Marrow Transplantation 2006 risk scoring schema (http://www.asbmt.org). Acute leukemia in first or second complete remission; CML in first chronic phase; Hodgkin's or non-Hodgkin's lymphoma in complete or partial chemotherapy sensitive remission, CLL in first remission, myelodysplastic syndrome or myeloproliferative disorder without excess blasts were considered standard risk and all others determined to be high risk at the time of transplantation. Additionally, univariate analysis was performed as supporting evidence using a non time-dependent non-weighted average CsA level from days 0 through day +14 post-transplantation so that the competing risk of death is minimized and hardly any GVHD events (n=3) were included prior to measurements; results were divided into categories of <200, 200–249, 250–299, 300–349, 350–399 and 400+ ng/ml. Tarone's test for trend was used to complete the univariate comparison of endpoints by CsA level (15). Cumulative incidence estimates are reported for acute GVHD, chronic GVHD, treating non-event deaths as a competing risk. Cumulative incidence estimates were also reported for non-relapse mortality (NRM) treating relapse as a competing risk (11). Kaplan-Meier curves were used to estimate the probability of overall survival (16).

RESULTS

Three hundred thirty seven patients were studied. Patient characteristics are summarized in Table 1. Of the 128 SIB transplants, 51 received myeloablative and 77 received nonmyeloablative conditioning. Among 209 dUCB transplants, 67 received myeloablative and 142 nonmyeloablative conditioning. The study groups were similar for year of transplant (p=0.32), gender (p=0.27) and recipient CMV serostatus (p=0.35). There were

significant differences among groups in diagnosis, disease risk, conditioning regimen, age, and ABO match. The day −1 mean CsA levels were 155 ng/ml (range, 107 –615 ng/ml) for myeloablative SIB, 197 ng/ml (range, 90–828 ng/ml) in nonmyeloablative SIB, 155 ng/ml (range, 71–920 ng/ml) in myeloablative dUCB and 181 ng/ml (range, 55 ->1000 ng/ml) in nonmyeloablative dUCB recipients (p=0.002). Thus, the proportion of patients who required dose adjustment prior to infusion of the allograft were 65% in myeloablative SIB, 51% in nonmyeloablative SIB, 79% in myeloablative dUCB and 59% in nonmyeloablative dUCB recipients.

The cumulative incidence of acute grade 2–4 GVHD was 35% (95% confidence interval [CI], 23–48%) for myeloablative SIB, 40% (95%CI, 29–51%) for nonmyeloablative SIB, 55% (95%CI, 42–68%) for myeloablative dUCB and 39% (95%CI, 31–47%) nonmyeloablative dUCB recipients (p<0.01). The cumulative incidence of NRM at 2 years was 14% (95%CI, 5–23%) for myeloablative SIB, 26% (95%CI, 16–36%) for nonmyeloablative SIB, 46% (95%CI, 33–59%) myeloablative dUCB and 25% (95%CI, 18–32%) nonmyeloablative dUCB recipients (p<0.01). The high risk of NRM in the myeloablative dUCB group is, at least in part, due to the low incidence of the competing risk, relapse. Overall survival at 5 years was 54% (95% confidence interval [CI], 36–68%) for myeloablative SIB, 41% (95%CI, 27–55%) for nonmyeloablative SIB, 44% (95%CI, 31–57%) for myeloablative dUCB and 34% (95%CI, 25–43%) for nonmyeloablative dUCB recipients (p=0.11).

Based on the background data, demonstrating lower CsA levels in the week prior to the development of acute GVHD (2), we felt that the weighted average was an appropriate method to study the effect of CsA on the development of GVHD. Thus, in the multivariable models, after adjusting for donor type and conditioning regimen we observed that every 50 ng/mL increase in the weighted average trough CsA level resulted in a 33% reduction in the risk of grade 2–4 (Table 2). We did not observe an effect of early CsA levels on the risk of chronic GVHD. Notably, higher weighted average CsA level also resulted in a 33% reduction in the risk NRM and 23% reduction in the risk of overall mortality (Table 2).

While we found higher CsA levels to be independently and significantly associated with improved outcomes, we expected the benefit would eventually plateau and studied whether there was a threshold above which further increases in the CsA level did not result in further improvement of outcomes. As summarized in Table 3, these data suggest that patient's CsA levels above the minimum target range (200 ng/mL) had lower incidence of acute GVHD and NRM and improved survival.

DISCUSSION

Our study demonstrated that higher weighted average CsA levels early after allo-HCT were associated with reduced risks of acute GVHD, non-relapse and overall mortality. In our transplant center, regardless of the donor type or intensity of the conditioning regimen, we initiate CsA immunoprophylaxis IV at day −3 with the goal of achieving target levels by the time of the infusion of the allograft. In order to achieve the target level prior to the infusion of the graft we measure the CsA level on day −1 and if the level was < 200 ng/mL promptly

adjust the dose. The rationale for this strategy was that the activation and expansion of alloreactive T-cells occurs early after the infusion of the graft. Thus, achieving therapeutic levels of CsA early after allo-HCT would be potentially protective from GVHD; findings were corroborated in murine models long ago (17). Our weighted average models confirmed the importance of the CsA level measured close to the development of acute GVHD as described in an early report that observed an association between CsA level < 200 ng/mL and a higher risk of developing acute GVHD in the following week (2). This study demonstrated that CsA trough levels < 200 ng/mL were associated with higher risk of GVHD. In contrast to our study, this early report was prior to the introduction of nonmyeloablative regimens and CsA/MMF immune suppression. Thus, ours report demonstrated that the importance of CsA levels extends to the current era where CsA/MMF regimens are widely used.

We also studied the effect of the non-weighted average of all CsA levels up to day +14 so that the competing risk of death is minimized and only a rare acute GVHD events prior to day +14 (n=3) were included; this method allowed actual cumulative incidence estimates by CsA level. In contrast, the time-dependent weighted average method up to day +30 that did not include any CsA measurements after the onset of acute GVHD; for example, we only considered CsA measurements up to day 18 if acute GVHD occurred on day +19. Thus, taken together the results by both methods provide good supporting evidence of the contribution of maintaining therapeutic CsA levels early after transplantation in reducing the risk of acute GVHD.

In our study, after adjusting for the CsA level, the risk of acute GVHD was similar whether patients received SIB or dUCB graft after a myeloabalative or nonmyeloablative conditioning. However, Ram et al (5) showed that higher calcineurin inhibitor (cyclosporine or tacrolimus) levels in the first 2 weeks after allo-HCT was associated with a lower risk of acute GVHD in nonmyeloablative, but not in myeloablative transplants. Similar to our strategy, in the nonmyeloablative setting they started CsA on day −3 at 5 mg/kg/day IV in 2 divided doses (in some cases at 5–6.25 mg/kg twice daily orally), while in the myeloablative setting CsA was started on day −1 at 3mg/kg/day IV. Notably, and similar to our study, in the nonmyeloablative setting they also observed a reduction in the risk of non-relapse and overall mortality with higher CsA levels. Kedmi et al (4) reported their institution's experience in which CsA was started at day −1 before 2003 and since 2003 it was started on day −4. They found the patients starting cyclosporine at −4 had less acute and extensive chronic GVHD. In a smaller cohort, Malard et al (6) found that a higher CsA level in the first week after allo-HCT was associated with lower risk of acute GVHD. In contrast to previous reports, our study also included recipients of dUCB transplantation that have been shown to have a high risk of acute GVHD (18). While these earlier studies used different conditioning regimens, donor types, methodologies to measure CsA levels and target trough levels, the overall conclusions have been consistent. Thus, our policy of consistent CsA initiation and dosing may explain, at least in part, the independent effect of CsA levels on the observed risk of GVHD regardless of the conditioning regimen intensity (5).

In summary, our data demonstrated that maintaining therapeutic CsA levels early after sibling and dUCBT transplantation contributes to a reduction of risk of GVHD and NRM,

and improved probability of survival. Our data and that of others (4, 5), support the initiation of a calcineurin inhibitor around day −3 prior to the infusion of the allograft. We speculate, that measuring the level on day −1 and adjusting dose up if clinically indicated to achieve therapeutic levels prior to the infusion of the allograft would be beneficial. However, an added implication of our findings is that for patients with CsA trough levels at or modestly above the target limit, in the absence of CsA related toxicity, dose reduction should cautious in order to avoid low or subtherapeutic drug levels which can increase the risks of acute GVHD and early mortality.

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Table 1

Patient, graft and transplant characteristics

Variables	Total
Number of patients	337
Year of transplantation	
2006	83 (24%)
2007	66 (20%)
2008	85 (25%)
2009	59 (185)
2010	44 (13%)
Median Age (range)	50 (15–74)
Male Gender	213 (63%)
Cytomegalovirus seropositive patient	209 (62%)
Disease	
Acute lymphoblastic leukemia	47 (14%)
Acute myeloid leukemia	126 (37%)
MDS/MPD/CML	55 (16%)
NHL/Hodgkins lymphoma	84 (25%)
Other	25 (8%)
High Risk Disease	178 (53%)
Prior Autologous Transplant	47 (14%)
Donor Type	
Sibling PBSC	128 (38%)
Double UCB	209 (62%)
ABO Match	
Match	127 (38%)
Minor mismatch	97 (29%)
Major mismatch	110 (33%)
Conditioning	
Myeloablative	118 (35%)
Nonmyeloablative	219 (65%)
Graft-vs.-host disease prophylaxis	
Cyclosporine A/mycophenolate mofetil	286 (85%)
Cyclosporine A/methotrexate	51 (15%)
Median Follow-up of Survivors (range)	2 years (0.8–5.2 years)

MDS, myelodysplastic syndrome; MPD, myeloproliferative disease; CML, chronic myeloid leukemia; NHL, non-Hodgkin's lymphoma, PBSC, peripheral blood stem cells; UCB, umbilical cord blood; HLAm, human leukocyte antigen.

* One patient received a 4/6 + 6/6 double umbilical cord blood graft.

Table 2

Multivariable analysis: time-dependent weighted average cyclosporine A levels (per 50 mg/ml) on outcome.

Outcomes and Variables	RR (95% CI)	P
GRADE II–IV ACUTE GRAFT-VS. HOST DISEASE		
Weighted Average cyclosporine A level	0.75 (0.62–0.90)	**<0.01**
Donor Type		
Myeloablative Sibling	1.0	
Nonmyeloablative Sibling	0.74 (0.31–1.78)	0.50
Myeloablative dUCB	1.57 (0.73–3.40)	0.25
Nonmyeloablative dUCB	1.02 (0.49–2.16)	0.95
CHRONIC GRAFT-VS. HOST DISEASE		
Weighted Average cyclosporine A level	0.90 (0.69–1.17)	0.42
Donor Type		
Myeloablative Sibling	1.0	
Nonmyeloablative Sibling	0.69 (0.27–1.78)	0.44
Myeloablative dUCB	1.63 (0.61–4.35)	0.33
Nonmyeloablative dUCB	0.35 (0.15–0.85)	**0.02**
Age at Transplantation		
15–34	1.0	
35–54	3.28 (1.31–8.23)	**0.01**
55+	6.34 (2.20–18.30)	**<0.01**
Patient Cytomegalovirus Serostatus		
Negative	1.0	
Positive	2.85 (1.39–5.83)	**<0.01**
NON-RELAPSE MORTALITY		
Weighted Average cyclosporine A level	0.75 (0.60–0.93)	**<0.01**
Donor Type		
Myeloablative Sibling	1.0	
Nonmyeloablative Sibling	4.01 (0.88–18.32)	0.07
Myeloablative dUCB	5.55 (1.30–23.73)	**0.02**
Nonmyeloablative dUCB	3.16 (0.73–13.63)	0.12
OVERALL MORTALITY		
Weighted Average cyclosporine A level	0.80 (0.67–0.95)	**0.01**
Donor Type		
Myeloablative Sibling	1.0	
Nonmyeloablative Sibling	1.89 (0.79–4.53)	0.15
Myeloablative dUCB	1.98 (0.85–4.60)	0.11
Nonmyeloablative dUCB	1.85 (0.83–4.12)	0.14
Patient Gender		
Male	1.0	
Female	1.49 (0.98–2.23)	0.07

dUCB, double umbilical cord blood.

Each 50 ng/ml increment of CsA level was tested for its impact on the outcome of interest using 70% overweighting for the pre-event samples.

Table 3

Univariate effect of the non-weighted average CsA level between day 0 and day +14 post-transplantation.

Outcomes	N	Day 100 Incidence (95% CI)	P – test for trend
GRADE II–IV acute GVHD			
Average cyclosporine A level in ng/mL			
<200	26	51% (40–62%)	**0.04**
200–249	62	47% (34–60%)	
250–249	93	47% (36–58%)	
300–349	91	47% (36–58%)	
350+	65	35% (25–45%)	
NON-RELAPSE MORTALITY			
Average cyclosporine A level in ng/mL			
<200	26	40% (20–60%)	**0.02**
200–249	62	36% (23–49%)	
250–249	93	25% (15–35%)	
300–349	91	25% (15–35%)	
350+	65	22% (13–31%)	
OVERALL SURVIVAL			
Average cyclosporine A level in ng/mL			
<200	26	38% (19–57%)	0.07
200–249	62	37% (24–51%)	
250–249	93	46% (33–59%)	
300–349	91	35% (23–47%)	
350+	65	46% (31–61%)	

GVHD, graft-versus-host disease.