Folinic Acid Supplementation in Higher Doses is Associated with Graft Rejection in Pediatric Hematopoietic Stem Cell Transplantation

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Folinic acid is widely used in hematopoietic stem cell transplantation (SCT), mainly to reverse antifolate effects of such drugs as methotrexate and cotrimoxazole but also empirically to reduce toxicity and support hematopoietic recovery. However, concerns have been raised in oncohematology about reduced curative rates associated with folinic acid administration. The clinical impact of folinic acid with regard to graft-versus-host disease (GVHD), relapse, and rejection in pediatric SCT is largely undetermined. In this single-center retrospective study we investigated folinic acid administration in 87 children undergoing SCT between 2007 and 2010. Data on folinic acid dosage and duration were analyzed along with SCT parameters using univariate and multivariate statistics. Folinic acid treatment was not correlated with relapse or GVHD grades $\geq$ II. However, significantly higher folinic acid doses until day +21 post-SCT had been administered to patients rejecting their grafts ($P < .005$). In a subanalysis of nonmalignant disease and reduced-intensity conditioning (RIC) SCTs, higher total folinic acid doses were found to be associated with rejection ($P = .015$ and $P = .026$). Multivariate analysis identified RIC (odds ratio, 19.9; $P < .01$) and an early total folinic acid dose of >185 mg/m^2 (odds ratio, 11.4; $P = .03$) as risk factors for graft rejection. Late folinic acid treatment had no impact on relapse, GVHD, and rejection. To conclude, administration of folinic acid in pediatric SCT seems safe in terms of relapse and GVHD. However, it should be carried out with caution, especially in patients with nonmalignant conditions and those receiving RIC to avoid graft rejection.

INTRODUCTION

Folinic acid is widely used to minimize the side effects of antifolate chemotherapeutic agents administered to patients suffering from hematologic and oncologic disorders. In allogeneic hematopoietic stem cell transplantation (SCT), folinic acid is used to reduce the toxicity associated with graft-versus-host disease (GVHD) prophylaxis with methotrexate (MTX) [1,2] and, ex juvantibus, to minimize side effects of the conditioning regimen. The beneficial effect of folinic acid administration in SCT (ie, reduced toxicity but preserved anti-GVHD effects of MTX) was demonstrated in dogs during the late 1970s [3,4]. Subsequently, the role of folinic acid in attenuating MTX-related side effects has been confirmed in clinical studies, also in non-SCT patients [2,5,6]. However, over several years it has been debated whether administration of folinic acid is necessary in SCT and if it might reduce the curative rate for hematologic malignancies by selectively rescuing leukemic cells [7-9]. The reduced antileukemic effect was also illustrated by Skäreby et al. [10], who described an increased risk of relapse in children receiving higher folinic acid doses after high-dose MTX treatment in the NOPHO 92 ALL protocol. In addition to administration of folinic acid as part of the conditioning regimen and on the following days, some cytopenic children have been given folinic acid post-SCT to reduce antifolinic acid effects by cotrimoxazole and to support hematopoietic recovery [1,11]. Because there are conflicting data concerning benefits and potential hazards associated with folinic acid, the aim of this study was to investigate the correlation between folinic acid treatment and graft rejection, relapse, and GVHD in children undergoing SCT.

METHODS

Patients

Between 2007 and 2010, 75 pediatric patients underwent SCT at our center. A number of patients underwent more than one SCT, with 87 total performed SCTs. Most patients had hematologic malignancies, but patients with nonmalignant conditions (ie, benign hematologic, metabolic, and primary immunodeficiency disorders) were also included (Table 1). The study was approved by the Regional Ethical Review Board in Stockholm.

SCT Procedure

The SCT procedure and conditioning regimens have been published previously [12], and treatment data are presented in Table 1. In most SCTs cyclosporine A and MTX (n = 52, 59.8%) were used as prophylaxis against GVHD. In the remaining SCTs, cyclosporine A and prednisolone (n = 16, 18.4%), tacrolimus and sirolimus (n = 13, 14.9%), or other combinations (n = 6, 6.9%) were administered. Antithymocyte globulin was applied when the stem cell source was an unrelated donor or in certain cases (n = 69, 79.3%). Cotrimoxazole was used as *Pneumocystis jiroveci* prophylaxis, in most cases 3 days per week during the first 6 months post-SCT.

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Table 1
Patient Characteristics (SCT, n = 87)

Characteristic	Value
Age, yr	7 (.25-19)
Sex, M/F	62 (71.3%)/25 (28.7%)
Diagnosis	
Nonmalignant	39 (44.8%)
Acute leukemia	29 (33.3%)
MDS	15 (17.2%)
Other malignancy	4 (4.6%)
Stage, early/late	53 (60.9%)/34 (39.1%)
Donor	
HLA-identical related	27 (31.0%)
MUD	41 (47.1%)
Mismatched*	19 (21.8%)
GVHD prophylaxis	
CsA + MTX	52 (59.8%)
CsA + prednisolone	16 (18.4%)
Tacrolimus + sirolimus	13 (14.9%)
Other	6 (6.9%)
Conditioning	
Cyclophosphamide	1 (1.1%)
fTBI + cyclophosphamide	20 (23.0%)
Busulphan + cyclophosphamide	31 (35.6%)
Fludarabine + cyclophosphamide	9 (10.3%)
Fludarabine + fTBI + cyclophosphamide	3 (3.4%)
Fludarabine + treosulphan	23 (26.4)
Antithymocyte globulin	69 (79.3%)
Nucleated cell dose, 10^8/kg (range)	4.9 (.2-34)
CD34 dose, 10^6/kg (range)	4.3 (.03-28.2)
Stem cell source	
BM/PBSC/CB	56 (64.4%)/13 (14.9%)/ 18 (20.7%)
Donor sex, M/F	51 (58.6%)/36 (41.4%)
Donor age, yr (range)	21 (0-52)
Acute GVHD	
0	43 (49.4%)
I	9 (10.3%)
II	25 (28.7%)
III-IV	10 (11.5%)
Relapse, yes/no	10 (20.8%)/38 (79.2%)
Rejection	11 (12.6%)
Retransplant	
First SCT	73 (83.9%)
Second SCT	12 (13.8%)
Third SCT	2 (2.3%)

MDS indicates myelodysplastic syndrome; HLA, human leukocyte antigen; MUD, HLA-A, -B, and -DR matched unrelated donor; CsA, cyclosporine; fTBI, fractionated total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; CB, cord blood.

* HLA-A, -B or -DR mismatched unrelated donor.

Folinic Acid Treatment

Patients receiving GVHD prophylaxis containing MTX on days +1, +3, +6, and +11 were given approximately 10 mg/m^2 folinic acid either daily from day −1 or day −1, +2, +4, +7, +8, +9, +12 and thereafter daily until an absolute neutrophil count >.5 × 10^9/L was found. In patients not receiving MTX, folinic acid in a similar dose and course was administered along with the conditioning regimen ex juvantibus on clinical indication to reduce toxicity and support hematopoietic recovery. Furthermore, folinic acid was also administered in some cytopenic patients at a later stage to reverse antifolic effects of cotrimoxazole and to support hematopoietic recovery. Folinic acid administration along with the conditioning regimen (ie, starting on day −1 to +14) or at a later stage (ie, day +15 and beyond) is referred to here as early and late folinic acid treatment, respectively.

Data Collection

Data on each patient were obtained from medical records and patient databases. The collected data included basic parameters (eg, height, weight, age, and sex), transplant characteristics (eg, diagnosis, conditioning, GVHD prophylaxis, donor source, and cell dose), and outcome parameters (eg, rejection, relapse, and GVHD reactions). Data on MTX, cotrimoxazole, and folinic acid dosage (eg, average daily dose, total dose) and treatment time were extracted from medication administration records. To verify the reproducibility of the abstracted data, 10 patients were selected randomly and reabstracted. Complete agreement between the two abstractions was confirmed.

Outcome Data

The endpoints chosen were GVHD, relapse, and rejection. Acute GVHD was graded following the international standard 1-4 [13]. Relapse was defined as increasing minimal residual disease, reaching diagnostic criteria. Rejection was defined as progressive mixed chimerism reaching >90% recipient-derived cells in the lineage of interest. GVHD, relapse, rejection, and survival data were locked for analysis on December 31, 2011.

Statistics

Relapse and GVHD were estimated using an estimator of cumulative incidence curves, taking competing events into consideration [14,15]. The logistic regression method was used in the predictive analysis for rejection. Factors analyzed were folinic acid dose and duration, age, diagnosis, donor, GVHD prophylaxis, nucleated cell dose, conditioning regimen, antithymocyte globulin, and stem cell source. Continuous variables were compared using the Mann-Whitney or Kruskal-Wallis test. Statistical significance was set at $P < .05$.

RESULTS

Folinic acid was administered early in 71 SCTs (81.6%), both early and late in 15 (17.2%) and only late in 1 (1.1%). The median treatment durations were 22 (range, 5 to 115) and 105 (range, 58 to 131) days for the early and late treatment stages, respectively. The total median folinic acid treatment dose per m^2 was 190 mg (range, 59.5 to 1111.8) for early treatment and 979 mg (range, 158.5 to 2194.5) for late treatment, whereas the average daily doses per m^2 were 10.7 mg (range, 8 to 35.8) and 13.8 mg (range, 5.3 to 24.1), respectively. In 30 SCTs (34%) folinic acid treatment was given without prior MTX administration.

Rejection

The total doses of early folinic acid associated with the risk of graft rejection in the analysis of all patients (median [range]) for rejection versus nonrejection were 241 [127-500] and 178 [31-1112] mg/m^2 ($P < .01$), whereas the daily average dose (median 10.5 [8.4-35.8] versus 10.3 [8.0-27.6] mg/m^2) and duration (median 23 [18-32] versus 21 [5-114] days) had no correlation with graft rejection. These findings remained unaltered when adjusted for MTX dosage (ie, MTX/CAL ratio > 1.0 versus <1.0).

To control for the possibility that clinicians increased folinic acid treatment due to worries about graft rejection, an analysis of the total folinic acid dose until day +21 post-SCT was performed. The correlation between a higher folinic acid dose and rejection was still confirmed (median 207 [127-442] versus 165 [0-301] mg/m^2, $P = .005$, Figure 1A). Because rejections are reported more frequently in nonmalignant and reduced-intensity conditioning (RIC) SCTs, further analyses were performed. A higher total dose until day +21 was seen in rejections compared with nonrejections among patients with nonmalignant diseases (median 210 [127-442] versus 186 [0-301] mg/m^2, $P = .015$, Figure 1B) as well as in patients with RIC (median 210 [127-442] versus 180 [62-301] mg/m^2, $P = .026$, Figure 1C).

Correlations between early total folinic acid dose and SCT parameters associated with graft rejection were identified: engraftment (ie, days to absolute neutrophil count >.5 × 10^9/L, r = .59, $P < .001$), total nucleated cell dose (r = −.21, $P = .005$), and CD34+ cell dose (r = −.36, $P = .001$). However, in a further multivariate analysis, only RIC (odds ratio, 19.9; 95% confidence interval, 2.22 to 177, $P < .01$) and an early total folinic acid dose >185 mg/m^2 (odds ratio, 11.4; 95% confidence interval, 1.24 to 104, $P = .03$) were identified as risk factors for graft rejection. The characteristics of patients with graft rejection are presented in Table 2.

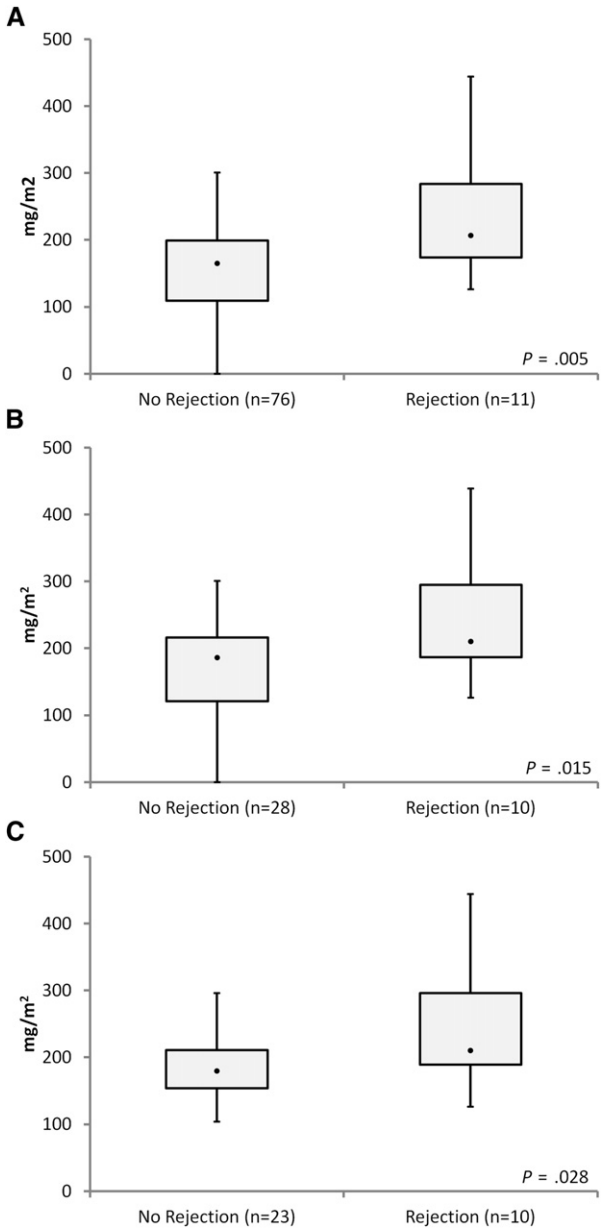

Figure 1. Folinic acid supplementation is associated with graft rejection. A higher total folinic acid dose until day +21 post-SCT was observed in the SCTs where graft rejection occurred (A). The correlation was also confirmed in patients with nonmalignant diseases (B) and reduced-intensity conditioning (C).

Relapse

Total dose, daily mean dose, and duration of early folinic acid treatment had no correlation with relapse in malignant disease. No impact of folinic acid treatment on relapse could be identified when adjusted for the MTX dosage (ie, MTX/CAL ratio $>$ 1.0 versus $<$1.0). Late folinic acid was not associated with an increased incidence of relapse.

GVHD

Total dose, daily mean dose, and duration of early folinic acid treatment had no correlation with the frequency of acute GVHD grade I, II, or III-IV. No effect of folinic acid treatment on GVHD could be identified on adjusting for MTX dosage (ie, MTX/CAL ratio $>$ 1.0 versus $<$ 1.0). Late folinic acid treatment was not found to be associated with an increased incidence of GVHD.

DISCUSSION

Folinic acid reduces mucosal and gastrointestinal side effects caused by MTX [1,2,6]. Moreover, folinic acid is sometimes used in a situation of poor hematopoietic recovery in hematologic patients, despite insufficient supporting data and, over the years, even contradictory research evidence [8,16]. However, reports raising concerns that folinic acid selectively rescues malignant cells have led some clinicians to restrict folinic acid usage [8-10].

The present study found no association between folinic acid dosage or treatment duration and relapse in malignant disease in either early or late administration. The same pattern was also seen when correlated with the administered MTX dose. The interpretation of the present data is that folinic acid might be used in pediatric patients undergoing SCTs due to malignant disease. The concerns about an increased relapse risk as a result of folinic acid rescue of malignant cells [9,10] do not seem to be valid in pediatric SCT patients who have received high-dose chemotherapy to achieve acceptable pre-SCT minimal residual disease and heavy SCT conditioning.

If folic acid rescued malignant cells and supported lymphocyte proliferation [8,17], one may hypothesize that donor-derived lymphocytes that mediate GVHD could benefit from the same supporting effect. Nevertheless, no association was found between folinic acid dosage and treatment duration and GVHD, after either early or late administration or when correlated with the administered MTX dose.

Patients rejecting their grafts, however, had received significantly higher early folinic acid doses (ie, starting at day -1 to $+14$) compared with patients without graft rejection. In the absence of engraftment or suspicion of threatening graft failure, folinic acid supplementation is easily continued, although its efficacy is non–evidence based to support hematopoietic recovery. To control for this possible confounder, we compared total doses until day $+21$ post-SCT, and the correlation between rejection and dose remained valid. Virtually all rejections occurred after SCTs for nonmalignant diseases and RIC SCTs, but also within these groups there was a correlation with the folinic acid doses. Obviously, these patients received less aggressive conditioning with the risk of leaving residual recipient marrow cells [18,19]. In an analogy with leukemic cells being rescued by folinic acid and lymphocytes promoted to proliferate [8,17], one can hypothesize that the residual recipient marrow cells might have a better chance to survive due to folinic rescue, with subsequent rejection as a result. This hypothesis of restored host immunity is supported by others describing beneficial effect of folinic acid on immune function, already at levels far inferior to those used in SCT [17,20]. It might also be assumed that folinic acid rescue can be even more pronounced in patients receiving low-dose or no MTX. Additionally, the known polymorphisms in the interconnected metabolic pathways of folinic acid should be taken into consideration. The administration of the same dose folinic acid to different patients may result in varying serum levels, with a possible subsequent effect on graft survival [21].

To summarize, the present study indicates that folinic acid is safe in pediatric SCT with regard to relapse in malignant disease and GVHD. However, the study suggests that

Table 2
Characteristics of Patients with Graft Rejection

UPN	Sex	Age (yr)	Diagnosis	Donor	Conditioning	GVHD Prophylaxis	CD34+ Dose ($\times 10^6$/kg)	Day ANC $> .5 \times 10^9$/L	Day of Rejection	Total Early Folic Acid Dose (mg/m^2)
1231	M	8	Fanconi	MUD	FLU+CY+ATG	CyA + prednisolone	.8	—	+25	332.9
1238a	M	1	Hurler	MUD	FLU+TREO+ATG	Tacrolimus + sirolimus	5.6	+20	+68	441.5
1238b	M	2	Hurler	MUD	FLU+CY+ATG	CyA	6.6	+19	+61	127.0
1230a	F	2	Kostman	SIB	FLU+TREO+ATG	CyA + prednisolone	.6	+54	+107	188.7
1269a	M	18	Kostman	SIB	FLU+TREO+ATG	CyA + MTX	1.0	+23	+49	241.5
1318b	M	11	CGD	MUD	FLU+CY+ATG	CyA + prednisolone	.1	+19	+34	190.5
1360a	M	1	SCID	MUD	FLU+TREO+ATG	CyA + prednisolone	.04	+25	+79	412.4
1390a	M	3	CGD	MUD	FLU+TREO+ATG	tacrolimus + sirolimus	4.3	+21	+80	203.9
1396b	M	.5	SCID	MUD	FLU+TREO+ATG	CyA + prednisolone	.14	+30	+25	406.5
1396a	M	.25	SCID	Mother	ATG	CyA + MTX + TcD	6.3	—	+28	500.0
1421a	M	4	JMML	MUD	BU+CY+ATG	CyA + MTX	5.9	+27	+48	185.8

UPN indicates unique patient number; ANC, absolute neutrophil granulocyte count; a, first SCT; b, second SCT; CGD, chronic granulomatous disease; SCID, severe combined immunodeficiency; JMML, juvenile myelomonocytic leukemia; MUD, HLA-matched unrelated donor; SIB, HLA-identical sibling donor; FLU, fludarabine; CY, cyclophosphamide; ATG, antithymocyte globulin; TREO, treosulphan; CyA, cyclosporine A; MTX, methotrexate; TcD, T-lymphocyte depletion.

administration of folinic acid should be limited and conservative in pediatric patients with nonmalignant conditions and those receiving RIC. Folinic acid should probably be avoided in patients with adequate folate levels in cases where MTX is omitted from the conditioning. Further studies, clinical as well as laboratory based, investigating the role of folinic acid in SCT biology are warranted.

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