Donor lymphocyte infusion for the treatment of leukemia relapse after HLA-mismatched/haploidentical T-cell-replete hematopoietic stem cell transplantation

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ABSTRACT

In this study, we tested the efficacy and safety of donor lymphocyte infusion (DLI) with granulocyte colony-stimulating factor (G-CSF) priming in patients who relapsed after haploidentical hematopoietic stem cell transplantation (HSCT). Twenty patients received DLI at a median of 177 days after HSCT. Eight patients survived in complete remission for a median of 1118 days. The 2-year probability of leukemia-free survival was 40%. Acute graft-versus-host disease (GVHD) grade 3-4 occurred in six patients after DLI. GVHD prophylaxis reduced the incidence of acute GVHD. Our primary data showed that G-CSF-primed DLI with GVHD prophylaxis was a potentially effective therapeutic option for patients who relapsed after haploidentical HSCT.

Key words: donor lymphocyte infusion, relapse, haploidentical, transplantation.

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Donor lymphocyte infusion (DLI) has been shown to exert a graft-versus-leukemia (GVL) effect and has been successfully used for the treatment of leukemia relapse in patients who have undergone HLA-matched, related or unrelated hematopoietic stem cell transplantation (HSCT).[1,2] Granulocyte colony-stimulating factor (G-CSF)-primed DLI might achieve a comparable or stronger GVL effect than that of the infusion of non-mobilized lymphocytes.[3] Furthermore, graft-versus-host disease (GVHD) prophylaxis might reduce the occurrence of fatal GVHD after DLI.[4] So far, there is little published information on the efficacy and toxicity of DLI in an HLA-mismatched setting.[5,6] In the present study, G-CSF mobilization and GVHD prophylaxis were applied to DLI in haploidentical transplantation, and the safety and efficacy of this treatment strategy was evaluated.

Design and Methods

Patients

Twenty patients who underwent haploidentical T-cell-replete HSCT between April 1, 2002, and May 1, 2005, and then relapsed were included. The characteristics of these 20 patients are summarized in Table 1.

Transplantation procedure

The transplantation procedure, including the conditioning regimens, donor priming, graft harvest and GVHD prophylaxis, was as described recently.[7]

Definitions

Hematologic relapse of leukemia after transplantation was defined as the recurrence of signs and symptoms of leukemia. In chronic myeloid leukemia (CML), cytogenetic relapse was defined as the recurrence of metaphases with the Philadelphia chromosome (Ph^+) without hematologic or clinical features of CML. Complete or partial responses were evaluable in patients who survived at least 30 days after DLI because the earliest examination of bone marrow morphology was done 4 weeks after the reinfusion. The severity of acute and chronic GVHD was determined according to the criteria described by Glucksberg *et al.*[8] However, GVHD was diagnosed as acute or chronic according to clinical features of the affected organs rather than time after DLI.

Table 1. Characteristics of the patients receiving donor lymphocyte infusion after HLA-mismatched hematopoietic stem cell transplantation.

No.	Gender	Age	Diagnosis	Stage of disease	HLA loci matched	Donor	Percentage blasts in BM at relapse	Time of DLI (day)	Outcome and cause of death	Follow-up (days)
1	male	50	ALL Ph$^+$	NR	5	sister	40%	280	RL	294
2	male	13	AML	RL	3	mother	6.5%	98/105	leukemia-free survival	1305
3	male	44	ALL	CR_1	5	brother	13.5%	89	RL	110
4	male	29	CML	AP	3	sister	7%	174	CR, infection	258
5	female	15	CML	BC	4	father	16%	46	RL	70
6	female	45	AML	MDS-RAEB	3	son	40%	134	non-remission	197
7	male	23	ALL Ph$^+$	CR_1	3	mother	20%	104	RL	427
8	male	23	ALL	NR	4	mother	9%	154	CR, infection	194
9	male	14	ALL	CR_1	3	mother	cytogenetic	201	leukemia-free survival	1214
10	male	23	ALL Ph$^+$	CR_1	4	mother	45%	346/374/405	CR, infection	655
11	male	19	AML	CR_2	3	mother	26%	135	CR, infection	259
12	male	14	ALL	CR_1	4	brother	13%	122/231/388	leukemia-free survival	632
13	male	6	ALL	CR_1	4	father	6%	143/198	leukemia-free survival	571
14	male	32	AML	CNSL	4	brother	22%	376	CR, GVHD	420
15	female	36	AML	MDS-RAEB	3	daughter	7%	570	leukemia-free survival	1019
16	male	17	AML	NR	4	father	21%	180	non-remission	237
17	female	28	ALL	CR_3	3	mother	15%	121	RL	246
18	male	32	CML	CP_1	4	father	cytogenetic	750	leukemia-free survival	859
19	male	20	ALL	CR_3	4	father	CNSL	414	leukemia-free survival	701
20	female	26	AML	NR	5	mother	pleural effusion	105	leukemia-free survival	400

GVHD: graft-versus-host disease; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myelogeneous leukemia; NR: non-remission; RL: relapse; CR: complete remission; AP: accelerated phase; BC: blast crisis; CNSL: central nerve system leukemia; MDS-RAEB: myelodysplastic syndrome-refractory anemia with excess blasts.

Donor lymphocyte infusion

Lymphocytes were obtained from cryopreserved G-CSF-mobilized peripheral blood (G-PB) or from fresh G-PB. The Peking University Review Board approved the cryopreservation, remobilization and infusion of the G-PB. All donors and patients signed a consent form. DLI doses were defined as CD3 cells per kilogram of recipient weight. Immunosuppressive agents were discontinued if patients were still receiving them when the leukemia relapse was diagnosed. However, after DLI 12 patients received cyclosporine A (blood concentration of 150-250 ng/mL for 2-4 weeks) or a low dose of methotrexate (10 mg once per week for 2-4 weeks) for prophylaxis against GVHD.

Statistical analysis

Time to GVHD after DLI was defined as the time from DLI to the onset of any grade of GVHD. The cumulative incidence of acute and chronic GVHD after DLI was calculated with competing risks of relapse and death. Distributions for time-to-relapse, time-to-complete remission, and actual leukemia-free survival were evaluated using Kaplan-Meier analysis. The surviving patients were followed up, and the results of follow-up were analyzed on June 30, 2006. Unless otherwise specified, all reported p values were based on two-sided hypothesis tests.

Results and Discussion

Relapse after transplantation

Twenty patients (Table 1) were diagnosed with relapse of leukemia at a median of 4.5 (1.5-35) months after HLA-mismatched HSCT without *in vitro* T-cell depletion. Before DLI, nine patients received one of the following chemotherapies: (i) methotrexate, 1 g; (ii) arabinoside (100 mg/m^2/day for 7 days) and daunorubicin (40 mg/m^2/day) for 3 days; (iii) mitoxantrone (4 mg/m^2/day on 1 and day 4) and etoposide (50 mg/m^2/day for 5 days), and (iv) fludarubicin (30 mg/m^2/day for 5 days). Two patients, one with Ph$^+$ acute lymphoblastic leukemia, the other with CML in blastic phase, were given imatinib (300-400 mg/day) for 22 or 89 days, respectively, and the patient with CML achieved complete remission. Nine patients received DLI without any prior intervention.

Response to donor lymphocyte infusion

A total of 27 infusions were given to 20 patients a median of 177 (46-750) days after HSCT. The cell subsets infused for transplantation and DLI are shown in Table 2. Eight patients survived without recurrence of their original leukemia through the end of follow-up, with a median follow-up of 1118 (range, 754-1468) days after HSCT and 808 days (range, 627-1388) days after DLI. The 1-year and 2-year probabilities of leukemia-free survival were 60 and 40%, respectively. Fifteen patients achieved complete

Table 2. Cells infused for transplantation and for DLI.

	Cell content in graft for stem cell transplantation						Cell content in products for donor lymphocyte infusion					
Pt No.	MNC (10^8/kg)	CD3 (10^8/kg)	CD4 (10^8/kg)	CD8 (10^8/kg)	CD34 (10^6/kg)	$CD3^-CD56^+$ (10^7/kg)	MNC (10^8/kg)	CD3 (10^8/kg)	CD4 (10^8/kg)	CD8 (10^8/kg)	CD34 (10^6/kg)	$CD3^-CD56^+$ (10^7/kg)
1	7.37	5.6	2.653	2.727	2.064	2.58	2	1.01	0.48	0.48	0.2	0.63
2	6.1	2.964	1.639	1.671	3.416	1.52	1.38	1.2	0.46	0.27	1.4	0.33
							0.25	0.13	0.06	0.04	0.23	0.46
3	7.29	5.016	2.263	1.749	1.272	2.14	5.95	4.39	2.48	1.92	1.2	0.16
4	10.65	2.88	1.604	1.455	8.326	4.72	3.5	1.42	0.83	0.66	1.015	1.82
5	11.64	1.666	1	0.804	9.69	2.22	1.0	0.28	0.15	0.12	1.1	0.17
6	6.08	2.1	1.14	0.84	2.4	0.92	1.0	0.56	0.32	0.22	0.69	0.22
7	7.51	2.73	1.45	1.25	3.52	1.66	1.0	0.47	0.25	0.19	0.23	0.12
8	6.31	2.07	1.2	0.75	1.18	1.91	1.0	0.33	0.22	0.12	0.028	0.13
9	5.43	1.4	0.93	0.51	0.82	0.78	3.93	1.46	0.90	0.76	0.98	0.35
							4.09	1.76	1.2	0.79	1.33	0.44
							3.0	1.40	0.76	0.58	0.69	0.22
10	7.47	1.21	2.83	4.25	2.18	2.59	3.26	1.13	0.57	0.51	1.798	1.87
11	7.245	1.3	0.7	0.49	1.07	3.22	3.07	1.27	0.64	0.46	0.768	1.76
							1.47	0.6	0.32	0.22	0.897	nd
							2.5	nd	nd	nd	nd	nd
12	8.64	2.20	2.87	1.10	3.00	2.00	1.0	0.23	0.17	0.11	0.54	0.23
13	7.26	1.8	1.034	0.61	4.94	1.84	0.2	0.08	0.05	0.03	0.092	0.01
							0.6	0.24	0.14	0.08	0.262	0.19
14	5.9	3.05	1.55	1.34	2.4	3.72	2.03	1.45	0.75	0.59	0.29	1.66
15	8.23	2.44	2.66	1.06	2.63	3.45	1.5	0.95	0.5	0.39	0.18	0.88
16	5.76	0.84	0.6	0.2	4.27	1.71	2.5	0.65	0.5	0.15	0.93	0.89
17	7.07	1.44	0.77	0.51	2.45	2.22	1.0	0.3	0.17	0.1	0.21	0.22
18	6.8	1	0.59	0.33	2.14	1.78	0.2	0.07	0.04	0.02	0.096	0.01
							1.4	0.48	0.28	0.14	0.68	0.06
19	8.58	2.87	2.4	1.63	2.78	1.82	1.23	.33	.41	0.16	0.82	0.24
20	5.8	1.63	0.94	0.56	1.11	1.19	1.0	0.47	0.27	0.16	0.2	0.02

MNC: mononuclear cells.

remission at a median of 289 (40-1388) days after DLI. Five of these fifteen patients were in the group that did not receive prophylaxis for GVHD after DLI, and the other ten patients were in the group that did receive prophylaxis. The blast count in bone marrow at the time of relapse was a risk factor influencing survival. The difference in bone marrow blast count at relapse between the group of patients who remained free of leukemia and the group of patients who subsequently died (6.0% versus 20.0%, respectively) was statistically significant (p=0.001). No extensive chronic GVHD occurred in the non-remission group; Seven of the 15 cases of extensive chronic GVHD occurred in the group of patients who achieved complete remission after DLI (p=0.046). However, this chronic GVHD was not found to influence survival.

GVHD and pancytopenia

Acute GVHD grades 1-2 occurred in five patients, and acute GVHD grades 3-4 occurred in six patients. In the initial nine patients studied, no GVHD prophylaxis was given after DLI. Five patients developed acute GVHD grades 3-4. In the eleven patients studied later, GVHD prophylaxis was applied, and only one developed acute GVHD grades III-IV (p=0.013, Figure 1). Chronic GVHD occurred in seven patients with a cumulative incidence of 64% at a median of 289 (40-1388) days after DLI, and six patients had the extensive type of GVHD.

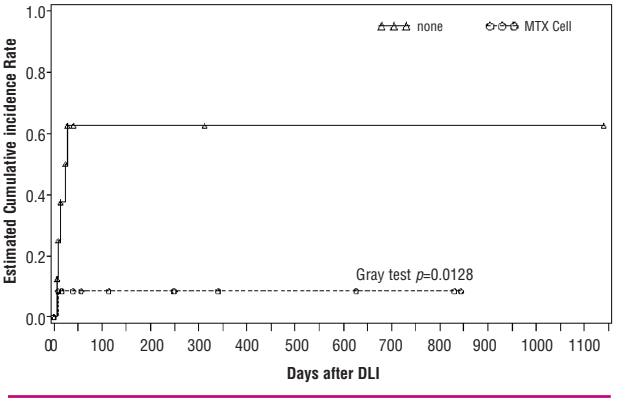

Figure 1. The influence of GVHD prophylaxis on occurrence of GVHD. In the initial nine patients studied, no GVHD prophylaxis was given after DLI. Five of these nine patients developed acute GVHD grades III-IV. In the eleven patients studied later, GVHD prophylaxis was given, and only one developed acute GVHD grades III-IV (p=0.0128)

The nadir of the white blood cell count occurred 48.5 (14-71) days after DLI, and that of the platelet count 48.5 (18-71) days after DLI. The white blood cell count decreased to less than 2.0×10^9/L in four patients and to

less than 1.0×10^9/L in one patient. The platelet count decreased to less than 50×10^9/L in seven patients and to less than 20×10^9/L in three patients, of whom two did not achieve remission and one had severe GVHD after DLI.

We recently we reported that the outcome of HLA-mismatched transplantation can be comparable to that of transplantation from HLA-identical siblings.[4] However, relapse is still a major complication and the best management of relapse of hematologic malignancy after allogeneic HSCT is uncertain, especially in those patients who have undergone an HLA-mismatched transplant.[1,5] We had studied the efficacy and safety of G-CSF-primed DLI with GVHD prophylaxis in HLA-identical HSCT.[3,4] Based on these findings, we began the study of G-CSF-primed DLI for patients who relapsed after HLA-mismatched HSCT.

In the present study, acute GVHD occurred in 11 of 20 patients after DLI, which was not higher than the rate in HLA-matched DLI. However, six patients developed acute GVHD grades 3-4 (most of them without GVHD prophylaxis), and three patients died of it. Hence, severe acute GVHD is a problem after HLA-mismatched DLI. Several strategies were applied to decrease its occurrence. First, GVHD prophylaxis was used in some of these patients. In HLA-matched transplantation, immunosuppressive agents were usually not given until GVHD occurred. In our initial cases of DLI, no prophylaxis was given, and there was a relatively high incidence of severe acute GVHD. We treated the second group of patients with cyclosporine A or methotrexate for 2-4 weeks. The incidence of severe GVHD was significantly reduced by GVHD prophylaxis and the therapeutic response remained acceptable. This suggests that prophylaxis for GVHD after DLI is a requisite in HLA-mismatched HSCT. The second strategy to decrease severe acute GVHD was to use G-PB instead of lymphocytes. There are many data indicating that the function of lymphocytes can be modified by G-CSF without this causing a loss of their antitumor alloreactivity.[4,9] The third strategy for reducing GVHD, sequential T-cell dose escalation, was applied in three patients.

Our primary data showed that treatment during an early stage of relapse was predictive for a higher possibility of complete remission and leukemia-free survival, indicating that DLI should be administered as soon as relapse is confirmed. Although many of the surviving patients developed extensive chronic GVHD, it was found that extensive chronic GVHD was not statistically associated with survival or death. Given the small number of patients in the present study, it is difficult to draw conclusions about the relationship between GVHD and GVL after DLI for HLA-mismatched patients.

In summary, this is the first study showing that DLI is a potentially effective therapeutic option for patients with a relapsed hematologic malignancy after HLA-mismatched HSCT. For DLI to be therapeutically effective in this setting, the incidence of severe acute GVHD must be decreased without abrogating the GVL effect.

Authors' Contributions

X-JH designed and monitored the research; DL analyzed the data and participated in this study; and all the other contributing authors participatedin the study.

Conflict of Interest

The authors reported no potential conflicts of interest.

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