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Etoposide With/Without G-CSF With Busulfan and Cyclophosphamide as Conditioning for Bone Marrow Transplantation

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To increase the efficacy of bone marrow transplantation (BMT), we have tried to add etoposide (VP-16) to busulfan/cyclophosphamide (BU/CY). Twelve patients received 16 mg/kg of BU and 120 mg/kg of CY with 15–30 mg/kg of VP-16. Another two patients received 5 μg/kg of G-CSF with 30 mg/kg of VP-16. Patients tolerated escalating doses of VP-16 without any significant hepatotoxicity. Their maximal level of bilirubin was 37.6 μmol/L (2.2 mg/dl), and there was no significant skin toxicity or mucositis. By contrast, two patients who received G-CSF with 30 mg/kg of VP-16 developed hyperbilirubinemia and veno-occlusive disease, which terminated this phase I study. VP-16 can be safely combined with BU/CY ≤30 mg/kg in three divided doses, and its effect on survival should be evaluated. G-CSF added to this regimen, however, should be used with great caution.

Key words: bone marrow transplantation, etoposide, G-CSF

INTRODUCTION

The combination of busulfan and cyclophosphamide (BU/CY) has been known to be an effective preparative regimen for bone marrow transplantation (BMT) for chronic myeloid leukemia (CML) and acute leukemia (AL). But when applied to high-risk patients such as those with CML in accelerated phase (AP), AL in first relapse, or more advanced stages, the results are discouraging. Accordingly, many manipulations have been tried to increase the efficacy of BU/CY, including adding total body irradiation (TBI) and etoposide (VP-16). Because of its effect against leukemic cells, VP-16 has been considered one of the most appropriate antileukemic agents to be added to BU/CY. Many phase I studies using VP-16 as part of the preparative regimen for BMT have been reported, and the maximal tolerable dose of VP-16, when added to the BU/CY regimen, has been reported to be 30–60 mg/kg, when given as a single dose [1–3].

Because several reports have documented the beneficial effects of separating the total dose of VP-16 into three to five divided doses, we divided administration of the dose into 3 days [4]. Since there has been no definitive phase I study using VP-16 in separated doses with BU/CY, except for the data presented by Jones and Santos [5], we have performed part of a phase I study. Because it was considered unethical to increase the dose of VP-16 to >30 mg/kg in total, however, we scheduled the addition of granulocyte-colony-stimulating factor (G-CSF) to increase the efficacy of the same treatment regimen.

MATERIAL AND METHODS

Patients

High-risk patients with myeloid leukemia who would undergo BMT [acute myelogenous leukemia (AML) in more than second complete remission (CR) and CML in more than AP] were eligible for the study. Patients with ALL were eligible only if they were not candidates for a preparative regimen that included TBI. Exclusion criteria included an aspartate aminotransferase (AST) level above normal before BMT, any history of drug-induced liver

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damage, or documented hepatitis C virus (HCV) infection. Informed consent was obtained from all patients who participated.

Preparative Regimen

Patients were scheduled for BMT with VP-16 added to the regular BU/CY regimen [6]. Busulfan was administrated orally to a total of 16 mg/kg in 4 days (days −8 to −5) and 60 mg/kg/day of cyclophosphamide was given for 2 days (days −3 and −2). Phenytoin and mesna were used to prevent seizure activity and cystitis.

VP-16 was initially administered intravenously at a dose of 5 mg/kg/day for 3 hr from day −4 to −2. If no life-threatening regimen-related toxicity (RRT) was observed in three or four cases, the dose was escalated by 2.5 mg/kg/day ≤10 mg/kg/day, for 3 days (30 mg/kg in total). No further escalation of VP-16 was permitted by the ethics committee of the institution, in accordance with Spitzer's data [1].

As the final step of the dose escalation study, G-CSF (filgrastim, Kirin Brewery Co., Ltd, Tokyo, Japan) was added to the preparative regimen at a dose of 5 μg/kg/day from day −10 to −2. Daily white blood cell (WBC) counts were obtained, and G-CSF was stopped if the WBC count exceeded 30.0×10^9/L. It was restarted when the count decreased to less than 10.0×10^9/L.

Prophylaxis of GVHD

Cyclosporine was started on day −2 at a dose of 5 mg/kg/day and given by continuous infusion until day 3, when the dose was reduced to 3 mg/kg/day infused in two divided doses. Methotrexate was given at 10 mg/m^2 on day 1 and 6 mg/m^2 on days 3 and 6 [7].

Supportive Care

All patients, except for the one with a history of allergic reaction to the medicine, received tosufloxacin and fluconazole for decontamination of the gut. Trimethoprim-sufamethoxazole was also used 3 times a week during the whole course of BMT. Acyclovir was used from day −5 to day 30 at a dose of 750–1,500 mg/body/day in three divided intravenous doses. Patients were managed in a highly powered filtrated room during the neutropenic period.

Regimen-Related Toxicity

Complete blood count (CBC) was obtained three times a week, and liver function tests twice a week. During the preparative treatment using G-CSF, the CBC was obtained daily. Abdominal ultrasound study was performed by a trained technician and doctors. Regimen-related toxicity (RRT) was graded in accordance with the proposal of Bearman et al. [8]. Veno-occlusive disease (VOD) was diagnosed according to the criteria of Jones et al. [9].

TABLE I. Patient Characteristics in BMT Study

UPN	Age/Sex	Primary disease	VP-16 dose (mg/kg/day)	G-CSF	aGVHD (grade)
53	27/F	CML/BC	5	−	0
54	17/F	AML/CR2	5	−	0
55	20/M	CML/CP2	5	−	0
58	35/F	CML/CP2	7.5	−	0
72	38/F	CML/AP	7.5	−	II
73	27/M	ALL/CR2	7.5	−	II
77	21/F	AML/CR2	7.5	−	I
89	40/M	CML/AP	10	−	IV
110	21/M	AML/CR2	10	−	0
112	45/M	AML/MDS	10	−	I
115	46/M	CML/AP	10	−	0
116	41/M	AML/MDS	10	+	0
118	18/F	AML/CR2	10	+	0

M/F, male/female; AML/MDS, acute myelogenous leukemia secondary to myelodysplastic syndrome; CR, complete remission; CP, chronic phasee; AP, accelerated phase; BP, blastic phase; VP-16, etoposide; aGVHD, acute graft-versus-host disease.

STOP Criteria

If two of three or three of six cases developed more than grade III RRT, graft failure, or bilirubin of >42.8 μmol/L (2.5 mg/dl) before day 20 or were diagnosed with VOD, the study was scheduled to be discontinued.

Statistical Analysis

Unpaired *t*-test was used to compare means. If the F-test was significant, the Wilcoxon rank-sum test was substituted for it.

RESULTS

Patients

All patients who participated in this study were high-risk patients with myeloid leukemias (AML 6, CML 6). One patient with ALL in the second CR also participated in this study because he was not a candidate for the regimen, including TBI. All patients received bone marrow from HLA identical siblings except for one who underwent autologous bone marrow transplantation (UPN 54 who received 5 mg/kg/day of VP-16). Their ages at the time of BMT were between 17 and 46 years (average 30.5 years), and 7 of them were male.

Their AST levels just prior to BMT were all within the normal range, and their pre-BMT bilirubin levels were <12.0 μmol/L (0.7 mg/dl). Two patients had never received blood products before BMT, and no patient had a history of previous infection due to hepatitis B virus or HCV (Table I).

Dose Escalation of VP-16

Patients tolerated the escalating dose of VP-16 well. The degree of mucositis and diarrhea did not reach signifi-

TABLE II. Side Effects and Outcome in BMT Study

UPN	Liver function tests BIL	AST	GGT	VOD	Stomatitis	Diarrhea (ml/day)	Outcome (days)
53	25.7 (1.5)	74	150	–	I	520	41R
54	12.0 (0.7)	27	NA	–	II	—	205R
55	17.1 (1.0)	14	40	–	I	—	+1,215
58	30.8 (1.8)	29	325	–	I	450	712R
72	17.1 (1.0)	43	213	–	I	—	+970
73[a]	37.6 (2.2)	342	113	–	II	2,035	104R
77	17.1 (1.0)	182	119	–	I	410	76R
89[b]	18.8 (1.1)	247	124	–	II	3,100	+515
110	12.0 (0.7)	20	27	–	I	420	+179
112	18.8 (1.1)	28	169	–	II	—	+165
115	13.7 (0.8)	58	602	–	I	1,065	+150
116	419.0 (24.5)	11	419	+	II	—	+130
118	46.2 (2.7)	43	292	–	II	—	+109

[a]Systemic adenovirus infection.
[b]Acute GVHD grade IV.
BIL, total bilirubin levels in μmol/L, parentheses in mg/dl; AST, aspartate aminotransferase (U/L); GGT, γ-glutamyl transferase (U/L); NA, not available; R, relapse; VOD, veno-occlusive disease.

cant levels, and in no case was it necessary to stop the dose escalation study up to the dose of 10 mg/kg/day for 3 days (Table I).

None of the patients developed VOD, even though one patient had a γ-glutamyltranferase (GGT) level of 602 U/L shortly after BMT. This elevation resolved quickly without any increase in bilirubin level. The maximal bilirubin level within 20 days after BMT never reached >37.6 μmol/L (2.2 mg/dl). One patient developed grade IV acute graft-versus-host disease (GVHD) that involved impaired liver function tests after day 14 (UPN 89). Another patient developed systemic adenovirus infection, including liver and renal damage, which started on day 17 (UPN 73) (Table II).

Mucositis occurred in all patients, but none developed more than grade II toxicity, and the volume of stool never exceeded 1,065 ml/day, except for two patients, one with grade IV acute GVHD and another who suffered from systemic adenovirus infection. Neither pulmonary nor neurological toxicity was observed, and there was no case of skin rash due to the medications.

Recovery of neutrophils was delayed (>50 days, to reach 0.5×10^9/L) in one patient, and delayed recovery of platelet count (>60 days, to reach 50.0×10^9/L) was observed in another.

Addition of G-CSF to BU/CY/VP-16

Two patients received G-CSF with the maximal dose of VP-16 but, because both developed hyperbilirubinemia of >42.8 μmol/L (2.5 mg/dl), the study was discontinued. Both patients also suffered from severe epigastric pain, which had started by the day of BMT, was accompanied by an increasing level of GGT, and required intravenous narcotics for >10 days to relieve the pain.

One patient (UPN 116) developed hyperbilirubinemia of ≤419.0 μmol/L (24.5 mg/dl) by day 11 with hepatomegaly and weight gain of more than 5%. There were no changes in transaminase levels or signs of other organ damage, however, and he responded dramatically to 1 mg/kg of prednisolone, with decreasing bilirubin and GGT levels. This patient has been exposed to the same medications included in this regimen on a separate occasion before BMT without any liver toxicity.

Another patient (UPN 118) developed hyperbilirubinemia of ≤46.2 μmol/L (2.7 mg/dl) and an increasing level of GGT without any significant increase of body weight, ascites production or documented hepatomegaly. Abdominal ultrasound examination also failed to show any abnormalities (Table II).

DISCUSSION

We have performed part of a phase I study of divided doses of VP-16 added to the BU/CY regimen in the setting of BMT because there were no definitive phase I data. We divided the total dose of VP-16 into three doses because it was shown that the activity of VP-16 against tumors is greater when administered in multiple rather than single doses [4]. Because it was considered unethical to increase the dose of VP-16 to >30 mg/kg in total, we then scheduled the addition of G-CSF to increase the efficacy of the treatment regimen.

Patients tolerated the same dose of VP-16 as did patients in previously published studies. However, the maximal level of bilirubin was significantly less than the reported data using a single dose of VP-16, which might be due to the separated dose schedule in our regimen. No patient had >37.6 μmol/L (2.2 mg/dl) of bilirubin, which made this regimen easier to use.

Spitzer et al. [3], for example, recently published their experience using VP-16 with BU/CY. The maximally tolerated dose of VP-16 could not be defined definitively from their data, but the medial peak bilirubin level of the patients was 70.1 μmol/L (4.1 mg/dl), and nine of 32 patients developed life-threatening or fatal hepatotoxicity while receiving 25 to 60 mg/kg of VP-16 with 12–16 mg/kg of busulfan and various doses of cyclophosphamide (60–200 mg/kg) [3]. Even though significant correlations were not observed between the dose of VP-16 and the incidence of side effects, the incidence of hepatotoxicity seemed unacceptably high for VP-16 to be used with BU/CY in their study design, and it seemed reasonable to set the maximal dose at 30 mg/kg in three divided doses in our study.

Jones and Santos [5] also reported the same regimen using VP-16 in three divided doses with BU/CY using 120 or 150 mg/kg of CY. Dose-limiting toxicity was

observed in 4 of 5 patients with 60 mg/kg of VP-16 using 120 mg/kg of CY and in 3 of 4 patients with 50 mg/kg of VP-16 using 150 mg/kg of CY. Jones and Santos [5] recommended to use 36 mg/kg of VP-16 with 150 mg/kg of CY, but the maximal bilirubin levels are not available. In comparison with their data, the dose schedule of our study might be too small for VP-16 and could have increased up to 50 mg/kg of VP-16 in total, which was not allowed by the institution.

G-CSF was then added to this regimen to drive the cells in G0 phase into G1 or to S phase during the preparative treatment, intending to increase the efficacy of chemotherapeutic agents and to decrease the chance of relapse of leukemia after BMT. Although either granulocyte- or granulocyte-macrophage-colony-stimulating factor or interleukin-3 (G-CSF, GM-CSF, or IL-3) can be used for this purpose, we chose G-CSF because of its availability to us [10]. There is also a report suggesting that G-CSF would enhance the expression of DNA topoisomerase II, which would augment the effect of VP-16 [11].

When G-CSF was added to the regimen, we did not predict any significant side effects, except for increased numbers of WBC during the treatment. However, two consecutive patients developed hyperbilirubinemia with significant epigastralgia, for which they needed continuous intravenous infusion of narcotics. Even though the possibilities of drug effects other than those of G-CSF and the preparative regimen could not be completely excluded, it seemed reasonable to warn that G-CSF added to the BU/CY/VP-16 regimen might be responsible for these side effects.

There are increasing numbers of reports of interstitial pneumonia caused by the addition of G-CSF to general chemotherapeutic agents [12,13]. It was speculated that the increasing number of WBC and/or increased synthesis of superoxide may be responsible for organ damage when chemotherapeutic agents are used simultaneously with G-CSF. The liver damage observed in this study might also be due to the same kind of effect of G-CSF added to that of the preparative regimen itself.

In conclusion, VP-16 can be safely added to the BU/CY regimen up to 30 mg/kg with less toxicity if the dose is divided over 3 days. If G-CSF is added to this regimen, however, the regimen should be used with great caution. It is too early to evaluate the outcome of the patients who participated in this study.

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