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Phase I Study of High-Dose Thiotepa With Busulfan, Etoposide, and Autologous Stem Cell Support in Children With Disseminated Solid Tumors

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Background. The aim of this phase I study was to define the maximum tolerated dose (MTD) of thiotepa (TT), administered with busulfan (BU) 480 mg/m^2 and etoposide 2,400 mg/m^2, followed by autologous bone marrow transplantation (ABMT) or peripheral blood stem cell transplantation (APBSCT) support in children with solid tumors either disseminated at diagnosis or after relapse. ***Procedure.*** Nineteen patients, between 2 and 16 years of age, received a high-dose chemotherapy regimen including escalating doses of TT starting from 150 mg/m^2. Subsequent dose escalation was determined by a modified Fibonacci scheme. Whenever one patient at one dosage level showed a grade III or grade IV reversible toxicity, additional patients were admitted (one by one) up to a maximum number of 6. Upon observing grade III or IV reversible toxicity in two or more systems, in 3 of the 6 patients, no further escalation was performed, and the corresponding dosage was taken as the MTD. WHO criteria were adopted to assess grade of toxicity. ***Results.*** All patients had hematological recovery; and neutrophils and platelet engraftment were observed after median times of 12 and 29 days from stem cell infusion, respectively. The MTD of TT was determined to be 750 mg/ m^2. At this level, 3 of 6 patients experienced grade III mucositis and/or grade III gastrointestinal toxicity. No patient died of treatment-related toxicity. ***Conclusions.*** A dose of 750 mg/m^2 TT is the MTD when it is associated with BU 480 mg/m^2 and etoposide 2,400 mg/m^2. This ablative regimen represents a feasible and tolerable combination for high-dose chemotherapy followed by hematopoietic stem cell rescue (HSCR). Phase II studies in children with poor-prognosis solid tumors are required to evaluate the effectiveness of this treatment. Med. Pediatr. Oncol. 33:450–454, 1999.

Key words: thiotepa; autologous stem cell transplantation; solid tumors

INTRODUCTION

The prognosis for children with disseminated solid tumors at diagnosis or after relapse remains poor despite the adoption of multimodal combined treatment. High-dose chemotherapy followed by autologous bone marrow transplantation (ABMT) or autologous peripheral blood stem cell transplantation (APBSCT) has been introduced as therapy of patients who failed conventional chemotherapy and for consolidation treatment of patients with chemosensitive solid tumors in first complete or partial remission considered at high risk of relapse [1,2]. The main aim of such therapy, not limited by hematological toxicity, is to combine agents with broad cytotoxic activity and nonoverlapping extrahematological toxicity.

Thiotepa (N,N′,N″-triethylenethiophosphoramide) and its metabolite tepa (N,N′,N″-triethylenephosphoramide) are polyfunctional alkylating agents synthesized by the American Cyanamid Company (Wayne, NJ) in 1952. These nonvesicant drugs can be administered by the intravenous (iv), intravesical, or intrathecal routes [3]. During early clinical development, TT was noted to be active against a wide variety of solid tumors, including those of childhood, such as neuroblastoma (NB), Wilms tumor, and central nervous system (CNS) tumors [3–6]. Because alkylating agents have demonstrated a logarithmic dose-response curve, they are ideal candidates for use during high-dose combination chemotherapy regimens [7]. Furthermore, etoposide, when associated with alkylating agents, has been documented to display a synergistic activity towards animal and human tumor cell lines [8]. Therefore, it is of interest to identify the maximum tolerated dose (MTD) of TT in combina-

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TABLE I. Characteristics and Outcome of Patients According to Thiotepa Dose Level*

Pt	Level (D)	TT mg/m^2	Sex	Age (years)	Disease	Status at HSCR	HSC source	Gr. III–IV tox.	FUP (months from BMT)
1	I (D)	150	M	6	ES	II CR	ABMT	NO	DOD (7∗)
2	I (D)	150	F	16	RMS	II CR	ABMT	NO	DOD (16∗)
3	I (D)	150	F	2	RMS	I CR	ABMT	NO	DOD (6∗)
4	II (2D)	300	F	12	ES	PR	ABMT	NO	NED (58+)
5	II (2D)	300	F	5	NB	I CR	ABMT	NO	NED (57+)
6	II (2D)	300	M	3	NB	PR	ABMT	NO	DOD (4∗)
7	III (3D)	450	M	5	NB	II CR	ABMT	NO	DOD (38∗)
8	III (3D)	450	F	2	NB	PR	APBSCT	NO	DOD (7∗)
9	III (3D)	450	M	6	NB	PR	APBSCT	NO	AwD (56+)
10	IV (4D)	600	F	3	ES	II CR	ABMT	NO	AwD (53+)
11	IV (4D)	600	F	4	NB	VGPR	ABMT	NO	AwD (51+)
12	IV (4D)	600	M	8	NB	PR	APBSCT	Gr. III M	DOD (51∗)
13	IV (4D)	600	M	3	NB	VGPR	ABMT	NO	NED (53+)
14	V (5D)	750	F	4	NB	VGPR	ABMT	Gr. III GI	NED (48+)
15	V (5D)	750	M	3	NB	PR	ABMT	NO	NED (40+)
16	V (5D)	750	F	3	NB	I CR	APBSCT	Gr. III GI/M	DOD (36∗)
17	V (5D)	750	M	3	NB	PR	APBSCT	NO	DOD (22∗)
18	V (5D)	750	M	5	NB	PR	APBSCT	NO	DOD (14∗)
19	V (5D)	750	F	2	NB	PR	APBSCT	Gr. III M	NED (45+)

*TT, thiotepa; HSCR, hemopoietic stem cell rescue; ES, Ewing sarcoma; RMS, rhabdomyosarcoma; NB, neuroblastoma; CR, complete remission; VGPR, very good partial remission; PR, partial remission; ABMT, autologous bone marrow transplantation; APBSCT, autologous peripheral blood stem cell transplantation; M, mucositis; GI, gastrointestinal; AwD, alive with disease; NED, alive with no evidence of disease; DOD, dead of disease.

tion with etoposide and other alkylating agents, insofar as its use in several solid tumors of childhood could improve antitumor efficacy and overcome tumor resistance.

Studies in adults have shown that the MTD of TT without stem cell rescue is 60 mg/m^2. The most common related toxicity of the drug at this dosage is severe, dose-dependent myelosuppression [9]. The Children's Cancer Group further demonstrated that the MTD without stem cell support in children is 65 mg/m^2 [10].

Infusion of hematopoietic stem cells makes it possible to overcome profound postchemotherapy myelosuppression, facilitating the use of higher dosages. The MTD of TT in combination with other drugs and stem cells rescue in adults was established at 600 mg/ m^2 [11]. The phase I study reported herein was designed to identify the MTD, defined by extramedullary organ toxicity, of TT in combination with busulfan (BU) and etoposide and followed by ABMT or APBSCT in children with solid tumors disseminated at diagnosis or who relapsed after conventional chemotherapy.

MATERIALS AND METHODS

Eligibility Citeria

Patients younger than 17 years of age with disseminated or relapsed solid tumors were eligible for the present study. Parents were required to give informed consent for transplantation and associated procedures. Children were eligible for this protocol if they had adequate cardiac (ejection fraction >45% by echocardiogram), hepatic (total plasma bilirubin $\leq$2 mg/dl and transaminases <2 times normal), renal (plasma creatinine $\leq$1.5 mg/dl), and pulmonary (forced vital capacity and forced expiratory volume $\geq$70% of predicted) function.

Patients

From April, 1992, to October, 1994, 19 children, 10 females and 9 males, with a median age of 5 years (range 2–16 years) were studied. The tumor types in this series were eight neuroblastoma (NB), three Ewing sarcoma (ES), and two rhabdomyosarcoma (RMS). The characteristics of the patients are summarized in Table I, where children are also stratified according to TT dose level. All patients had previously undergone extensive treatment prior to entry in this study. They had all previously received conventional chemotherapy containing alkylating agents. Surgery for the primary tumor had been performed in 7 cases. Only two patients (Pts 1 and 2) received radiation therapy (LRT) to their primary abdominal (Pt 2) or pelvic (Pt 1) mass. Patients' follow-up was updated as of December 31, 1997.

Stem Cell Collection

Either ABM or APBSC was accepted as stem cell sources for marrow reconstitution after dose-intensive chemotherapy. Prior to starting chemotherapy, either bone marrow harvest under general anesthesia or mobilization of peripheral blood was performed. Hematopoietic progenitors were mobilized in the peripheral blood with one or two cycles of cytoreductive-mobilizing cy-

clophosphamide (4 g/m^2) and etoposide (600 mg/m^2) followed by granulocyte colony-stimulating factor (G-CSF; Filgrastim) until stem cell collection. A minimum of 1×10^8/kg body weight of bone marrow mononuclear cells or 2×10^6/kg body weight of peripheral blood CD34+ was required. Stem cells were subsequently frozen at −120°C after admixture with 10% dimethylsulfoxide (DMSO) and stored in liquid nitrogen.

Drug Administration

The BU-E-TT regimen consisted of BU 480 mg/ m^2 given orally every 6 hr in 16 divided doses (days −9 to −6), etoposide 800 mg/m^2/day in 0.9% sodium chloride at a concentration of 0.4 mg/ml by 24 hr continuous infusion (days −5 to −3), and TT given in a solution of 0.9% of sodium chloride iv over 2 hr, in two doses, the first one starting 12 h after completion of the etoposide infusion (day −2). BU levels were measured, and drug dosage eventually adjusted, only in children less than 3 years of age according the experience of M.B. Regazzi and colleagues [12,13].

Transplant Procedures

Autologous bone marrow in 12 cases and peripheral blood progenitors in 7 cases were infused 36 hr after the last dose of TT. Day 0 designates the day of stem cell reinfusion.

Supportive Care

Central venous access via a single-lumen or multilumen silastic catheter was placed in all patients. Patients were isolated in laminar air-flow single rooms equipped with high-efficiency particulate air (HEPA) filters. In all patients, G-CSF at a daily dosage of 5 μg/kg iv over 1 h was administered starting on day +5 and was discontinued when the peripheral absolute neutrophils count (ANC) exceeded 2×10^9/liter. Platelet and packed red cell transfusions were used routinely to maintain a platelet count $>20 \times 10^9$/liter and a hemoglobin level >8 g/dl. All blood products were irradiated to a dose of 20 Gy before transfusion, and CMV-seronegative patients were transfused with seronegative blood products. Patients received acyclovir 250 mg/m^2 iv every 8 hr on days −1 to +21 and subsequently orally up to day +90 for prevention of herpes symplex infection. Before the ablative regimen, all patients received dental treatment consisting of oral hygiene improvement and necessary dental restorative procedures, whereas during aplasia all cases rinsed with 0.12% alcoholic chlorhexidine mouthwash three times daily for as long as they could. Patients who developed grade II or worse oral mucositis received tablets containing polymyxin E, tobramycin, and amphotericin B until the mucositis return to grade I [14]. Empirical broad-spectrum antibiotic therapy was started when children became febrile, and antifungal therapy was employed in the presence of clinical evidence of fungal infection or fever persisting after 3 days of antibiotic therapy. *Pneumocystis carinii* pneumonia prophylaxis was obtained with oral cotrimoxazole (6–8 mg/kg), starting from the day of engraftment until 6 months after transplantation. Total parenteral nutrition (TPN) was given through the central line during periods of inadequate enteral nutrition.

Study Design

The first dosage level of TT was at 150 mg/m^2 based on the following considerations. First, the highest dose of TT administered in adults, without stem cell transplantation, had been 60 mg/m^2 [3]; second, pediatric patients usually tolerate an average of 1.2 times (min–max.: 0.75–2.80) the MTD in adults [15]; third, stem cell support, eliminating problems related to myelotoxicity, allows one to at least double drug dosage. Subsequent dose escalation was determined by a modified Fibonacci scheme [16]. Whenever at one dosage level one patient showed a grade III or grade IV reversible toxicity, additional patients were admitted (one by one) up to a maximum of 6 patients. Upon observation of grade III or IV reversible toxicity in two or more systems, in 3 of the 6 patients, no further escalation was performed, and the corresponding dosage was taken as the MTD. On the basis of these criteria and considerations concerning the increment of drug dosage, the second TT dose level was 300 mg/m^2 (100% increment), the third was 450 mg/m^2 (50% increment), the fourth was 600 mg/m^2 (33% increment), and the fifth was 750 mg/m^2 (25% increment).

Toxicity and Engraftment Definition

WHO criteria were adopted to assess toxicity grade. Venoocclusive disease (VOD) of the liver was diagnosed and graded according to the criteria defined by Shulman and Hinterberger [17]. To assess toxicity, daily blood counts and chemical profiles of liver and renal function were performed from day −9 to hematological recovery. Appropriate tests and investigations were employed when indicated to evaluate organ toxicity. White blood cell (WBC) engraftment was defined as the first of 3 consecutive days when the ANC was $>0.5 \times 10^9$/liter and platelet engraftment as the first of 3 consecutive days when the unsupported platelet count was $>50 \times 10^9$/liter.

RESULTS

Toxicity

All patients were valuable for toxicity. Table II shows the details of the toxicity observed and subdivided according to the TT dose level.

Hematological toxicity. All patients experienced severe neutropenia (ANC $<0{,}5 \times 10^9$/liter) and severe thrombocytopenia (platelet count $<20 \times 10^9$/liter). Median (min–max) numbers of platelets and packed red cell

TABLE II. Toxicity Grade According to Each Thiotepa Dose Level*

	150 mg/m^2				300 mg/m^2				450 mg/m^2				600 mg/m^2				750 mg/m^2			
Grade	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Heart		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bladder		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Kidney		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Liver		—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—
CNS		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oral mucosas		—	—	—	1	2	—	—	—	3	—	—	1	2	1	—	1	3	2	—
GI		—	—	—	2	1	—	—	2	1	—	—	1	1	—	—	—	4	2	—
Lung		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

*CNS, central nervous system; GI, gastrointestinal.

transfusions of 5 (1–19) and 4 (3–15), respectively, were transfused during the period of aplasia. Median (min–max) durations of neutropenia $<0.5 \times 10^9$/liter and thrombocytopenia $<50 \times 10^9$/liter were 12 days (9–34) and 29 days (16–86), respectively. No hemorrhage occurred.

Infectious complications. During neutropenia, all patients experienced fever for a median (min–max) duration of 7 (2–19) days. Fever was related to septicemia in three children (*Staphilococcus epidermidis*). The outcome of fever was favorable in all cases.

Gastrointestinal toxicity. Digestive toxicity was moderate and consisted of vomiting in 8 patients and nausea for a median (min–max) duration of 5 (1–8) days in 5. Grade III diarrhea was observed in 2 patients only at higher doses of TT. Grade III stomatitis occurred in 3 of 19 patients for a mean duration of 4 days. Narcotics were used in only 1 case for 5 days.

Hepatic toxicity. Grade I hepatotoxicity was observed in 3 patients, consisting of cytolysis (raised transaminases levels). Two cases of moderate hepatic VOD disease were observed; both resolved rapidly without any treatment.

Skin toxicity. Cutaneous toxicity was moderate and consisted of acute erythrodermia in 10 patients (IV and V dosage levels). Secondary melanodermia was observed in 6 patients (V dosage level). No life-threatening toxicity occurred.

Outcome

As of December, 1997, 6 patients are alive with no evidence of disease (NED) and 3 with disease after a median (min–max) follow-up of 53 (40–58) months. Thirteen patients relapsed after a median (min–max) of 15 (1–29) months (Table I). No long-term extramedullary toxicities were detected in all surviving patients or in the subgroup treated at MTD.

DISCUSSION

Incremental cytoreduction of malignant cell is achieved after dose escalation of chemotherapeutic agents in certain childhood solid tumors. However, even though myelosuppression can be avoided by the transplantation of hematopoietic stem cells, dose escalation is limited for many drugs because of a variety of other organ toxicities. It is, therefore, of crucial importance for the elaboration of effective treatment schemes to identify the MTD of antineoplastic drugs used alone or in combination with other agents. In this phase I study, the aim was to find the MTD of TT when it was associated with BU and etoposide in a high-dose regimen for children with solid tumors. We observed the association of oral mucositis and gastrointestinal toxicity as both the most common and the dose-limiting toxicity of 750 mg/m^2 TT. All children but one enrolled in the 4D and 5D thiotepa dose level were affected by disseminated neuroblastoma and received the same front-line chemotherapy; so we believe that prior treatment does not affect the results of our study.

The second most common toxicity we observed was hepatic toxicity, with mild elevation of hepatic enzymes in 5 of 19 patients. Of these 5 patients, only 2 showed evidence of jaundice, with a bilirubinemia level up to 2 mg/dl, ipoalbuminemia, increment of body weight >5% from basal level, and ascites; this finding was most probably attributable to the use of BU, it was always reversible and never life-threatening. Our results with high-dose combination therapy using these three drugs are comparable in terms of toxicity to results reported in the literature and confirm that TT, when combined with other alkylating agents, radiotherapy, and/or etoposide, has an MTD of 750–900 mg/m^2. In fact, Kamani et al. [18] found that, with the two-drug association of TT and etoposide combined with total body irradiation (12 Gy) in children with NB, the TT MTD was 750 mg/m^2 when patients received allografts and 900 mg/m^2 when they were given ABMT. In agreement with this, Grill et al. [19] reported the association of TT at 900 mg/m^2 with BU at conventional doses to have a severe toxicity in a phase II study on children with ependymomas.

CONCLUSIONS

TT at a dose of 750 mg/m^2 associated with BU 480 mg/m^2 and etoposide 2,400 mg/m^2 represents a feasible

and tolerable combination for high-dose chemotherapy followed by hematopoietic stem cell rescue. On the basis of the present results, a multicenter phase II study has been started in Italy. The aim is to evaluate the efficacy of this high-dose regimen, which associates the two alkylators, BU and TT, which have very similar dose-response curves and excellent penetration into the CNS, with etoposide. The last drug is a synergistic topoisomerase II inhibitor, which also shows good penetration across the blood–brain barrier, so that the excellent distribution of these three drugs in the CNS makes possible a phase II study not only in children with disseminated solid tumors but also in those patients with primary CNS tumors or patients who develop brain metastases.

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