A Phase I Trial of Amifostine (WR-2721) and Melphalan in Children with Refractory Cancer

Peter C. Adamson,[1] Frank M. Balis, Jean E. Belasco, Beverly Lange, Stacey L. Berg,[2] Susan M. Blaney,[2] Catherine Craig,[2] and David G. Poplack[2]

Pediatric Branch, National Cancer Institute, Bethesda, Maryland 20892 [P. C. A., F. M. B., C. G., S. L. B., S. M. B., D. G. P.], and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104 [J. E. B., B. L.]

ABSTRACT

Melphalan has a steep dose-response curve, but the use of high doses results in unacceptable myelosuppression. Strategies to circumvent this dose-limiting myelosuppression would allow for the administration of higher, more effective doses of melphalan. Amifostine (WR-2721) has been shown in preclinical studies to protect the bone marrow from the myelotoxicity of melphalan, and in clinical trials, to protect from the myelotoxicity of other alkylating agents. A Phase I trial of the combination of amifostine and melphalan was performed in children with refractory cancers to: (a) define the acute toxicities of amifostine and its maximum tolerated dose (MTD); and (b) to determine whether the dose of melphalan could be safely escalated when administered in combination with amifostine. Amifostine was administered i.v. as a 15-min infusion 30 min before melphalan. The starting dose of amifostine was 750 mg/m², with planned dose escalations in 30% increments. Melphalan was administered as a 5-min infusion using the previously defined MTD in heavily pre-treated patients, 35 mg/m², as the starting dose. The dose of melphalan was escalated by 30% increments. Nineteen patients, ranging in age from 3 to 24 years (median, 15 years), were entered on trial. The dose of amifostine was escalated to 2700 mg/m², which is approximately 3-fold higher than the adult recommended dose, without reaching a MTD. Fifteen patients experienced nondose-limiting (<25%), transient decreases in blood pressure after the amifostine infusion. Other nondose-limiting toxicities of amifostine included mild nausea and vomiting, flushing, anxiety, diarrhea, and urinary retention. Six patients, three each at the 2100 and 2700 mg/m² amifostine dose levels were treated with an escalated dose of melphalan (45 mg/m²). All of these patients experienced grade 4 neutropenia (< 500/mm³), and five of six patients had grade 4 thrombocytopenia. The duration of this dose-limiting myelosuppression exceeded 7 days in four of six patients. Although no dose-limiting (grade 3 or 4) toxicity was attributed to amifostine, significant anxiety and reversible urinary retention occurred at the two highest amifostine dose levels. A dose of 1650 mg/m² for pediatric Phase II trials is recommended. High doses of amifostine, however, do not appear to allow for escalation of melphalan beyond its single agent MTD of 35 mg/m².

INTRODUCTION

Melphalan has limited activity against childhood cancers when administered at standard doses of 20–35 mg/m². However, at doses of 45 mg/m² in previously untreated patients (1) and 65–180 mg/m² in autologous bone marrow transplant preparative regimens (2), melphalan has a broad range of activity with complete responses observed in patients with rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, acute lymphoblastic leukemia, Wilms' tumor, Hodgkin's disease, and osteogenic sarcoma (2). The use of melphalan at these higher effective doses, however, is limited by its significant myelosuppressive effect.

Strategies to circumvent this dose-limiting myelosuppression would enable the use of higher and more effective doses of melphalan.

The use of colony-stimulating factors, an approach that has been successful with other anticancer drugs, did not prevent severe myelo-suppression from melphalan, a stem-cell poison (3). An alternative approach is to administer a chemoprotective agent. Amifostine (WR-2721) is an organic thiophosphate that selectively protects against the cytotoxicity of alkylating agents. In preclinical studies, amifostine protects the bone marrow from the myelotoxicity of melphalan (4), and in clinical trials it appears to circumvent the myelotoxicity of other alkylating agents (5–10). Treatment of patients with amifostine before melphalan administration may allow for the safe escalation of melphalan doses beyond those currently tolerable.

We performed a Phase I trial of amifostine and melphalan in children with refractory cancers. The objectives of the trial were to determine the acute toxicity and define the MTD[3] of amifostine in pediatric patients and to determine the MTD of melphalan when administered in conjunction with amifostine.

MATERIALS AND METHODS

Drug. Amifostine and melphalan were provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Amifostine was administered as a 15-min i.v. infusion. Fifteen min after completion of the infusion, melphalan was administered i.v. over 5 min.

Patient Eligibility. Patients <25 years of age with histologically confirmed cancer refractory to conventional therapy were eligible for this trial. Patients must have recovered from the toxic affects of prior therapy before receiving amifostine/melphalan. All patients had adequate hepatic and renal function as defined by a serum bilirubin <1.5 mg/dl, serum transaminases <2 times the upper limit of normal, and a serum creatinine level <1.5 times normal for a patient's age. Patients were required to have a granulocyte count >1500/mm³ and a platelet count >100,000/mm³.

Before entry into study, informed consent was obtained from the patient or from his/her parent in accordance with individual institutional policies.

Study Design. The primary objectives of the Phase I trial were (a) to define the acute toxicities and the MTD of amifostine in children receiving an i.v. dose administered over 15 min; and (b) to determine if amifostine administration before i.v. melphalan allowed for dose escalation of melphalan beyond 35 mg/m². A minimum of three patients evaluable for toxicity were treated at each dose level.

The starting dose of amifostine was 750 mg/m² (80% of the adult recommended dose) with dose escalations in increments of 30%, rounded to the nearest 50 mg. If no acute dose-limiting toxicity, as defined below, was observed in a cohort of three patients, the amifostine dose was escalated to the next higher dose level. If acute dose-limiting toxicity was observed in one of three patients, three additional patients were studied at that dose level. The highest dose of amifostine tolerated by three of three or by five of six patients in the appropriate cohort was defined as the amifostine MTD. Toxicity was graded according to the National Cancer Institute-Cancer Therapy Evaluation Program (CTEP) common toxicity criteria. In clinical trials of amifostine in adult patients with cancer, acute hypotension was a significant toxicity (6, 11, 12). The current trial, therefore, defined dose-limiting hypotension as a >25% decrease in systolic or diastolic pressure that did not improve with administration of an i.v. fluid bolus or did not resolve (<25% decrease in systolic or diastolic pressure from baseline) by the time of melphalan administration.

Table 1 *Patient characteristics*

Characteristics	
No. of patients (no. of courses)	
Evaluable for response	19 (21)
Evaluable for amifostine toxicity	19 (21)
Evaluable for melphalan toxicity	18 (20)
Male/female	14/5
Age (yr)	
Range	3–25
Median	15
Diagnoses	
Osteosarcoma	7
Ewing's sarcoma	3
Primitive neuroectodermal tumor	2
Rhabdomyosarcoma	2
Primitive sarcoma of bone	1
Synovial cell sarcoma	1
Undifferentiated sarcoma	1
Lymphoma	1
Hepatoblastoma	1
Type of prior therapy - no. of patients	
Chemotherapy only	11
Chemotherapy and radiotherapy	8
Immunotherapy	3
Median no. of prior regimens (range)	4 (1–7)

Blood pressure was monitored with an automated sphygmomanometer (Dinamap; Critikin, Inc., Tampa, FL) every 15 min before the start of the amifostine infusion to determine the baseline blood pressure, then at 3–5-min intervals for 30 min starting with the amifostine infusion, and then at 15-min intervals for at least 1 h.

The starting dose for melphalan was 35 mg/m^2, the previously defined MTD in heavily pretreated pediatric patients, with planned escalations to 45 and 60 mg/m^2. Attempts were made to escalate the dose of melphalan when a cohort of three patients pretreated with the same dose of amifostine did not experience dose-limiting myelosuppression. Hematological dose-limiting toxicity was defined as grade 4 myelosuppression of >7 days duration, or an absolute granulocyte count <1500 cells/mm^3 or a platelet count of <100,000/mm^3 28 days after drug administration. The MTD for melphalan was defined as the dose level immediately below the dose level at which two patients of a cohort of three-six patients treated at the amifostine MTD experienced dose-limiting toxicity.

Patients were monitored with complete blood counts initially twice weekly and then at least every other day when neutropenic. Serum electrolytes, creatinine, calcium, phosphorus, uric acid, liver function tests, and urinalysis were monitored weekly, and patients were followed closely for clinical signs of toxicity. In patients with measurable disease, other laboratory or radiological examinations pertinent to tumor response were obtained before each cycle. Cycles were repeated at 21-day intervals. Patients were removed from the study if they experienced unacceptable grade 3 or 4 toxicity or if

objective disease progression was noted after one or more courses of amifostine/melphalan.

RESULTS

A total of 19 patients, 3–24 years of age (median, 15 years) were entered on study. All patients were evaluable for acute amifostine toxicity, and 18 patients were fully evaluable for melphalan toxicity. One patient died of progressive disease within 4 weeks of drug administration and was, thus, not fully evaluable for hematological toxicity of melphalan. The characteristics of the evaluable patients are listed in Table 1. All had been heavily pretreated with multiple chemotherapeutic regimens and, in many cases, radiation therapy.

The dose levels of amifostine studied and the number of patients treated at each dose level are listed in Table 2. No patient experienced acute dose-limiting toxicity attributable to amifostine. The majority of patients treated with amifostine doses $\geq$1000 mg/m^2 experienced nondose-limiting decreases in blood pressure (Fig. 1), but the degree of hypotension did not appear to be dose related at the higher dose levels. Nadir blood pressure occurred toward the end of the 15-min infusion, at a median of 13 min. One patient treated with 1000 mg/m^2 of amifostine experienced a nadir in blood pressure 100 min after the start of the infusion, which resolved with i.v. fluid administration. All other patients had return of blood pressure to normal by the time of melphalan administration. Other nondose-limiting amifostine toxicities included mild nausea and vomiting (16 patients), flushing (15 patients), anxiety (2 patients each at 1650, 2100, and 2700 mg/m^2), diarrhea (1 patient at 1650 mg/m^2), and urinary retention (2 patients at 2100 and 1 patient at 2700 mg/m^2).

The hematological toxicity of melphalan is shown in Table 2. For patients treated with 35 mg/m^2 of melphalan, there was a trend toward increasing myeloprotection with increasing doses of amifostine. The median nadir absolute neutrophil count increased from 153 to 752/mm^3 as the amifostine dose was escalated from 750 to 1650 mg/m^2 (Table 2).

The dose of melphalan was escalated to 45 mg/m^2 after three patients pretreated with 1650 mg/m^2 of amifostine did not develop dose-limiting myelosuppression. Six patients, three each at the 2100 and 2700 mg/m^2 amifostine dose levels, respectively, were treated with 45 mg/m^2 of melphalan. All of these patients experienced grade 4 neutropenia, and five of six patients had grade 4 thrombocytopenia. The duration of the dose-limiting myelosuppression exceeded 7 days (dose limiting) in four of these six patients.

Two objective responses were observed on this trial. A 15-year-old male with recurrent metastatic Ewings sarcoma, treated with 35

Table 2 *Hematologic toxicity*

Melphalan dose (mg/m^2)	Amifostine dose (mg/m^2)	No. of patients with hematologic DLT/no. of patients treated	Absolute neutrophil count		Platelet count	
			Nadir count average (cells/mm^3) [range]	Day of nadir median [range]	Nadir count average ($\times$ 10^3/mm^3) [range]	Day of nadir median [range]
35	750	2/3	153 [0–442]	13 [9–14]	61 [9–61]	16 [12–17]
35	1000	2/3	295 [60–528]	15.5^a [14–17]	12 [8–22]	15.5 [14–17]
35	1300	2/3	482 [39–864]	18 [14–22]	29 [3–80]	15 [15–17]
35	1650	0/3	752 [200–1752]	16 [15–18]	69 [38–113]	17 [14–19]
45	2100	3/3	103 [0–280]	14 [11–14]	8 [6–12]	14 [13–15]
45	2700	2/3	144 [0–432]	13 [12–14]	18 [2–35]	14 [12–17]

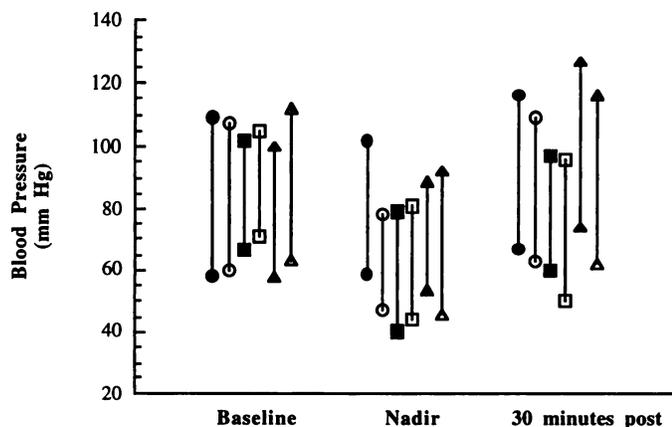^{a} One patient received G-CSF after 7 days of neutropenia.

Fig. 1. Average blood pressure (systolic top point and diastolic bottom point) in groups of patients treated with 750 (●), 1000 (○), 1300 (■), 1650 (□), 2100 (▲), and 2700 (△) mg/m² of amifostine administered i.v. over 15 min. Nadir blood pressures occurred at a median of 13 min after the start of the amifostine infusion.

mg/m² melphalan after 750 mg/m² of amifostine, and a 17-year-old female with refractory osteosarcoma of the jaw, treated with 45 mg/m² melphalan after 2100 mg/m² of amifostine, had partial responses (≥50% reduction in the sum of the product of the two longest perpendicular diameters of all measurable tumors and no appearance of new lesions for 4 weeks). Both patients had progressive disease after their second course of amifostine/melphalan.

The inability to safely escalate the melphalan dose despite an amifostine dose that was approximately 3-fold higher than the adult recommended dose (910 mg/m²), coupled with the anxiety and urinary retention observed with amifostine doses exceeding 2000 mg/m², led to termination of the trial. The recommended pediatric dose of amifostine for future Phase II trials is 1650 mg/m².

DISCUSSION

The protective capacity of thiol-containing compounds against radiation-induced tissue damage has been recognized for more than 40 years (13). Amifostine, developed by the United States Army as a radioprotective agent during the 1960s, was later found in preclinical studies to protect normal tissue from the effects of alkylating agents (14). The drug has subsequently been undergoing clinical evaluation as a radio- and chemo-protectant agent. Amifostine is a prodrug that is dephosphorylated extracellularly to its active metabolite, WR-1065, which preferentially accumulates in normal tissues (15). Within minutes of amifostine administration, WR-1065 achieves high concentrations within the bone marrow but it disappears rapidly such that by 2.5 h, WR-1065 is essentially cleared from the marrow (16, 17). To maximize the marrow protection of amifostine from alkylating agent toxicity, an agent with a short half-life is theoretically advantageous.

Melphalan, similar to other alkylating agents, has a steep dose-response curve, but its use is limited by dose-related myelosuppression. In one pediatric trial, approximately 40% of heavily pretreated patients experienced grade 4 hematological toxicity when melphalan was administered at a dose of 35 mg/m² (1). Other pediatric Phase I-II trials have administered melphalan at doses of 30–45 mg/m², with the higher dose producing unacceptable hematological toxicity in pretreated patients (18, 19). The pharmacokinetics of melphalan (2) are particularly favorable for use in conjunction with amifostine. Its short half-life (64 ± 9 min) will result in low plasma concentrations at a time when the marrow concentrations of WR-1065 have also diminished. This, in addition to the demonstrated clinical activity of melphalan when used at high doses and the preclinical data demonstrating

the chemoprotective effects of amifostine when combined with melphalan, made the combination of amifostine/melphalan a rational choice of agents to test the possibility of escalating the dose of an alkylating agent beyond currently tolerable doses by administration of a chemoprotective agent.

The most serious toxicity noted in adult patients receiving amifostine has been hypotension, occurring in 5% of patients treated with doses between 25 and 1330 mg/m² (12). No relationship between dose and hypotension has been observed. Therefore, most adult Phase II trials have proceeded by using 740–910 mg/m² of amifostine, a dose range considered tolerable (5, 8, 20). This pediatric Phase I trial determined that children appear to tolerate a higher dose of amifostine than do adults. Nondose-limiting hypotension did not appear to be dose related, as shown in Fig. 1. Hypotension resolved within 30 min in all patients except one child treated who experienced a late decrease in blood pressure 100 min after amifostine administration. Nausea and vomiting were usually abrupt in onset but short lived and appeared to be controlled with the administration of anti-emetics. Although no dose-limiting toxicity of amifostine was observed on this trial, problematic adverse toxicities appeared to cluster at the higher dose levels (2100 and 2700 mg/m²). Two toxicities not previously described that occurred at these dose levels were acute anxiety and urinary retention. The urinary retention observed in three patients resolved spontaneously within 4 h of amifostine administration. On the basis of these findings, we would recommend an amifostine dose of 1650 mg/m² for future pediatric trials.

In clinical trials in adult patients combining amifostine with standard doses of either cis-platinum or cyclophosphamide, there were significant increases in nadir hematological parameters (5–10). One trial has suggested that the MTD of carboplatin may be increased from 400 to 500 mg/m² with the administration of two consecutive doses of 740 mg/m² of amifostine (21); a Phase II study is further evaluating this possibility. The current pediatric Phase I trial was not designed to detect differences in the duration of myelosuppression after amifostine/melphalan, but rather to determine if a chemoprotective agent could be used to improve the therapeutic efficacy of an alkylating agent by allowing administration of a higher dose of drug. In this group of heavily pretreated patients, despite doses of amifostine 3-fold higher than doses used in adults, the dose of melphalan could not successfully be escalated above 35 mg/m². In five of the six patients treated with 45 mg/m² of melphalan, the ensuing myelosuppression was severe, with grade 4 toxicity lasting longer than 7 days in the majority of patients.

Amifostine can safely be administered to pediatric patients in doses up to 2700 mg/m². At these high doses, however, undesirable toxicities (anxiety and reversible urinary retention) are observed, whereas with doses of 1650 mg/m² or less, toxicity appears minimal. Despite high doses of amifostine, the dose of melphalan could not be escalated beyond its currently defined MTD.

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