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A phase I trial of continuous-infusion cyclophosphamide in refractory cancer patients*

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Summary. Cyclophosphamide demonstrates enhanced tumoricidal activity with decreased bone marrow toxicity when given on a divided-dose schedule in certain animal models. A total of 22 patients presenting with refractory metastatic cancer were treated in a phase I trial of continuous infusion of cyclophosphamide over 96 h. Granulocytopenia of <500/μl that lasted for >14 days or thrombocytopenia of <25,000/μl that lasted for >14 days was the target dose-limiting toxicity in the absence of nonhematologic grade 4 toxicity. The maximal tolerated dose was 7 g/m^2. Three patients died. Of 21 evaluable patients, 9 responded, including 8/9 who had experienced disease progression during prior oxazaphosphorine-containing combination chemotherapy. Clinically meaningful responses were observed in patients who had demonstrated clinical resistance to an oxazaphosphorine drug given at lower doses.

Introduction

Cyclophosphamide, an oxazaphosphorine derivative of bis-2-chloroethyl nitrogen mustard, is the most widely used alkylating agent. Cyclophosphamide undergoes extensive metabolism. The parent transport form displays low alkylating potential that produces a relatively specific tumoricidal activity and a therapeutic ratio that is impressive for an antineoplastic drug [3]. Granulocytopenia is the dose-limiting toxicity in clinical trials, but certain nonmyelosuppressive toxicities, cystitis and myopericarditis, become prominent at higher doses [11, 13].

Cyclophosphamide is active against proliferating and nonproliferating cancers, as are most alkylating agents. Unlike most alkylating agents, it is more cytotoxic to proliferating cells or tumors [5]. Efforts to improve the therapeutic/toxic ratio by schedule manipulations generally result in an improved therapeutic index in mice treated on multiple dosing schedules [14, 22, 23], although not in rat sarcoma [2]. The in vivo preclinical studies of multiple-dose cyclophosphamide in mice demonstrate a substantial increase in therapeutic ratio that is maintained over a broad dose range [14, 22, 23], resulting from both an increased cytotoxic effect and decreased toxicity to sensitive normal tissues. Decreased peak levels achieved using an intermittent schedule lower the cardiotoxicity of high-dose cyclophosphamide [21] and were first used clinically at the Royal Marsden Hospital in autologous bone-marrow-transplant conditioning regimens [20]. Using these observations as a basis, we began a phase I clinical trial of continuous-infusion cyclophosphamide in cancer patients as a prelude in including such a schedule in a high-dose combination regimen for autologous bone marrow transplantation.

Patients and methods

In a phase I study, patients presenting with unresectable or metastatic cancer received a continuous i.v. infusion of cyclophosphamide over 96 h; the starting dose was 2 g/m^2 per course (500 mg/m^2 i.v. daily over 24 h for 4 days). Mesna was given at the same dose as cyclophosphamide for 5 days as a continuous i.v. infusion to any patient who exhibited ≥50 red blood cells/μl urine. Mesna was used in the same solution. When 2/3 patients at a given dose level required mesna, all patients treated at subsequent dose levels received mesna. Infusions of cyclophosphamide (and mesna, when used) were prepared in normal saline and were changed daily. In all, >98% of cyclophosphamide's activity persists after 24 h in 0.9% saline solutions [4].

Doses were escalated at 1-g/m^2 increments, and dose escalation continued until 2 of 3–5 patients/dose level experienced dose-limiting toxicity (DLT). DLT was defined as irreversible grade 3 or any grade 4

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Table 1. Patients' characteristics

Patient	Age (years)	Diagnosis	Dose		Duration of Toxicity (days)		WHO grade		Infection	Response
			(g/m^2)	(No.)	ANC (<500)	Platelets (<25,000)	Cardiac	Hematuria		
1	43	SCLC	2	(2)	0	0	0	0	0	DP
2	60	DWDLL	2	(1)	14	14	0	0	0	MR
3	46	SCC anus	2	(1)	0	0	0	1	0	DP
3	46	SCC anus	3	(1)	8	0	0	1	0	DP
4	31	Melanoma	3	(2)	7	0	0	1	0	DP
5	33	Leiomyosarcoma	4	(4)	0	0	0	0	0	PR
6	39	Leiomyosarcoma	4	(2)	0	0	0	0	0	SD
7	70	Colon carcinoma	4	(1)	10	0	0	0	+	DP
8	49	SCLC	4	(2)	13	0	0	0	0	PCR
9	40	SCC anus	6	(2)	19	19	0	1	0	PR
10	42	CML	6	(1)	–	–	0	1	0	DP
11	29	Breast carcinoma	6	(2)	11	0	0	1	0	DP
12	47	Breast carcinoma	6	(3)	7, 14, 35	0	0	1	0	PR
13	41	Breast carcinoma	6	(1)	10	0	0	0	0	DP
14	29	DLCL	7	(1)	21	7	0	0	+	PR
15	34	FSCL	7	(1)	12	7	0	1	+	DP
16	18	Burkitt's lymphoma	7	(1)	10	9	0	0	+	CR
17	47	Breast carcinoma	7	(1)	31+	31+	0	0	Died	PR
18	61	FSCL	7	(1)	13	7	3	2	Died, +	IE
19	35	Thymoma	7	(1)	13	0	0	0	Died	DP
20	40	Breast carcinoma	7	(1)	0	3	3	3	+	PR
21	59	DLCL	8	(1)	19	19	0	0	0	CR
22	42	SCLC	8	(1)	19	0	0	0	0	DP

ANC, Absolute neutrophil count; DP, disease progression; MR, minimal response; SD, stable disease; PR, partial response; CR, complete response; PPR, pathologic partial response; PCR, pathologic complete response; IE, inevaluable; NSCLC, non-small-cell lung cancer; SCC, squamous-cell cancer; DWDLL, well-differentiated lymphocytic lymphoma; CML, chronic myelogenous leukemia; DLCL, diffuse large-cell lymphoma; SCLC, small-cell lung cancer; SNCL, small noncleaved lymphoma; FSCL, follicular small cleaved-cell lymphoma

toxicity (WHO grading). Myelosuppression was dose-limiting only if it lasted longer than 14 days (dose escalation along with autologous bone marrow support was planned if myelosuppression was the DLT). Perphenazine or metaclopramide was used as an antiemetic (no barbiturate or steroid was given). The dose level preceding the DLT was defined as the maximal tolerated dose (MTD).

Eligibility criteria included an age of >17 years; a CALGB performance status of <2; levels of SGOT, bilirubin, serum creatinine, and blood urea nitrogen that were <1.5 times the normal values; a WBC of >3,000/μl and a platelet count of >100,000/μl (unless disease-related); normal cardiopulmonary status; a creatinine clearance of >60 cm^3/min; the failure of any curative or palliative standard therapy; institutional review board approval; and signed informed consent. All patients had undergone prior chemotherapy and must have demonstrated disease progression during their last therapy, which must have been completed by ≥3 weeks prior to the beginning of the cyclophosphamide infusion. Daily blood counts and urinalysis were carried out in the hospital, and hematuria had to be ≤grade 1 before discharge. Outpatient management required biweekly blood counts and prompt reporting and admission for fevers of >38° C. After a septic death occurred at 7 g/m^2, all subsequent patients were hospitalized until granulocytopenia had resolved.

Patients were eligible for repeated treatments once myelosuppression had resolved (average, 4–6 weeks). Except in patient 2, who received a second cycle of therapy at 3 g/m^2, no dose escalation was implemented. Subjects 3, 5, and 9 had undergone pelvic irradiation >6 prior weeks in each case. No patient had received prior chemotherapy consisting of melphalan, chlorambucil, nitrosoureas, or mitomycin. All subjects were evaluable for nonmyeloid toxicity, and 21/22 were evaluable for all toxicities and for response. Complete responses (CR) or partial responses (PR) required either the total disappearance of or a reduction of >50% in the bidimensional area of all measurable sites of disease, respectively, for at least 1 month after the start of chemotherapy.

Results

A total of 22 patients aged a median of 42 years (range, 18–71 years) received 34 cycles (range, 1–5) of continuous-infusion cyclophosphamide (Table 1). One subject received a second cycle of therapy at the next highest dose level (from 2 to 3 g/m^2) because no significant toxicity occurred after his first cycle. All patients on this study had received prior chemotherapy and had exhibited progressive malignant disease during the most recent regimen prior to the present study.

Toxicity

Myelosuppression of granulocytes (≤500/μl) and/or platelets (<25,000/μl) for ≥14 days was dose-limiting. Both of the patients who were treated at 8 g/m^2 developed myelosuppression that continued for 19 and 21 days, respectively. Thus, 7 g/m^2 cyclophosphamide given as a continuous 96-h i.v. infusion was considered to be the maximal tolerated dose (MTD) the present study, which accepted significant myelosuppression.

Granulocytopenia of <500/μl developed in 5/8 patients who were treated at doses of ≤4 g/m^2 and in 13/13 evaluable subjects who were treated at higher doses at between 7 and 10 days following the onset of therapy. Thrombocytopenia of <25,000/μl developed in 2/13 patients at doses of ≤6 g/m^2 (both had previously undergone pelvic irradia-

tion) and in 6/9 subjects who were treated at doses of 7–8 g/m^2. A 47-year-old woman who received three cycles of therapy developed progressively longer periods of myelosuppression, suggesting a cumulative effect.

Myelosuppression resolved in all but three cases. The only patient who had exhibited a WBC of <3,000/μl and a platelet count of <100,000/μl had refractory chronic myelogenous leukemia (CML) with <500 granulocytes/μl and <25,000 platelets/μl at the time of treatment with 6 g/m^2 cyclophosphamide. His peripheral blast count fell somewhat and he continued to receive other therapy. A 35-year-old man presenting with thymoma died during neutropenia when he refused further supportive care because of unequivocal disease progression. A 46-year-old woman presenting with breast cancer died of fungal sepsis on day 31, after Stevens-Johnson syndrome that developed due to B-lactam antibiotics had aborted bone marrow recovery and caused secondary bone marrow aplasia.

Infectious complications increased markedly at 7 g/m^2. Whereas 1/13 patients who were treated at lower doses exhibited a severe but reversible infection, all 9 subjects who were treated at 7 or 8 g/m^2 required hospitalization and antibiotics. A 34-year-old man presenting with refractory non-Hodgkin's lymphoma (NHL) developed pneumonia but recovered. A 61-year-old man presenting with NHL developed fatal. *Escherichia coli* sepsis after a prior colostomy site had dehisced. The woman who developed Stevens-Johnson syndrome died of candidal sepsis.

Mesna was required for 1 patient at 6 g/m^2 and for 9/9 subjects at higher doses. Asymptomatic hematuria developed in 3/10 individuals who were treated with mesna and in 7/12 patients who were not. Only one subject developed grade 3 hematuria (clots), which resolved entirely on hydration.

Cardiac toxicity was observed in two patients who were treated at 7 g/m^2. A 40-year-old woman presenting with breast cancer developed clinical congestive heart failure with dyspnea at rest and pedal edema. Her radionucleotide ventriculogram (RVG) showed a decrease from 55% to 37% in her left ventricular ejection fraction. She responded clinically to digoxin and furosemide, which were later discontinued without subsequent recurrence of symptoms. No repeat RVG was obtained. A 61-year-old man exhibiting NHL and a history of hypertension developed recurrent supraventricular tachycardia during an episode of febrile sepsis. His arrythmia was controlled with electrical cardioversion and verapamil. An echocardiogram showed that his left ventricular function had not changed from the normal pretreatment value. In five of six patients receiving doses of 7 and 8 g/m^2 (including the two described above), baseline and follow-up evaluations of the left ventricular function revealed no change after cyclophosphamide treatment.

Response

Of 21 evaluable patients, 9 showed a complete (3) or partial response (6) to continuous-infusion cyclophosphamide despite documented progression of cancer during the most recent chemotherapy; 8 of these 9 had exhibited progressive disease during previous oxazaphosphorine therapy (8, cyclophosphamide; 1, ifosfamide). Three of four evaluable patients presenting with NHL responded, two of them completely. Three responders went off protocol onto other therapies (autologous bone marrow transplant, irradiation, and salvage chemotherapy).

Patient 15 exhibited disease progression during cyclophosphamide treatment and declined further therapy. An autopsy on patient 18, who died of sepsis, revealed a small, residual focus of lymphoma in the hilum. All other sites of preexisting generalized lymphadenopathy and massive abdominal and retroperitoneal adenopathy contained no lymphoma.

Three of five patients presenting with breast cancer showed brief (1–3 months) partial responses. One of two subjects presenting with small-cell lung cancer achieved a pathologic complete response of endobronchial disease following two cycles of continuous-infusion cyclophosphamide after having exhibited disease progression during standard-dose cyclophosphamide, doxorubicin, and vincristine. He subsequently received chest irradiation and was clinically free of tumor on his death (no autopsy was performed) 2 years later of a myocardial infarction. Two of seven patients displaying other tumor histologies showed brief partial responses.

Discussion

Intensification of the dose of many antineoplastic agents produces enhanced tumor cytotoxicity [10]. For alkylating agents such as cyclophosphamide, this dose-response effect is often log-linear. In high-dose combination chemotherapy (>60 mg/kg or 1.875 g/m^2), cyclophosphamide-containing regimens produce a high response rate in either previously treated or untreated patients [9, 16]. Single-agent studies of high-dose cyclophosphamide provide evidence of a high response rate in previously untreated patients (reviewed in [8]) and in subjects exhibiting refractory lymphomas (78% in 14 patients) [7] but little evidence of response in refractory solid tumors, even when prior therapy involved single-agent alkylating agents [17].

The effect of schedule on the therapeutic efficacy of alkylating agents, including cyclophosphamide, is much less clear [22]. The rationale for multiple doses of high-dose cyclophosphamide evolved from animal and human experience with the cardiotoxic effects of the drug [21]. Cyclophosphamide induces its own metabolism, increasing the peak level and area under the curve (AUC) and decreasing the plasma half-life, but the levels of active species do not necessarily change, although this finding is disputed [18, 24]. Several preclinical models show an enhanced therapeutic effect for intermittent administration of cyclophosphamide [14, 22, 23]. The finding of different results in different species could be due to many factors, the foremost of which involves differences in the pharmacokinetics between species [2]. Thus, this topic (schedule-dependent efficacy) is extremely difficult to evaluate.

In this study of continuous-infusion cyclophosphamide, the MTD was 7 g/m^2. Grade 4 myelosuppression was the

dose-limiting toxicity. The duration of 14 days was arbitrary but was set to enable us to evaluate nonmyelosuppressive toxicities, since the intention was to incorporate continuous-infusion cyclophosphamide into a preparative drug combination for autologous bone marrow transplantation along with other drugs given by continuous infusion in an effort to lessen toxicity through individual drug dosing on the basis of pharmacokinetic parameters. Regrettably, these pharmacology studies were not functional at the time of this trial. The MTD was identical to that previously found using pulse therapy [20].

In the present study, myelosuppression was severe but reversible. At the MTD (240 mg/kg over 4 days), bone marrow reinfusion did not shorten myelosuppression, consistent with the bone-marrow progenitor cell-sparing effect seen in vitro with 4HC-purged human bone marrow [6]. Nevertheless, the high incidence of sepsis remains the major cause of morbidity and mortality, and the amelioration of myelosuppression with human hematopoietic cytokines should be considered in future trials of high-dose cyclophosphamide.

The major nonmyelosuppressive toxicity was hematuria. Cystitis was reversible and required no specific therapy. The introduction of mesna at doses of >6 g/m^2 may have prevented more severe complications, although this cannot be stated conclusively. Myocardial toxicity due to high-dose cyclophosphamide has been well described [15, 19]. Primate studies show that schedule is an important factor in cardiac toxicity, since divided doses were less cardiotoxic than the same total bolus dose [21]. In one of six patients evaluated in the present study, a decrease in the left ventricular ejection fraction was measured at 3–4 weeks after cyclophosphamide therapy. Further dose escalation will be required to establish whether continuous infusion is a less cardiotoxic schedule of cyclophosphamide administration.

Responses were seen in 43% of patients. Eight of nine responders had previously demonstrated tumor growth while receiving oxazaphosphorines (cyclophosphamide or ifosfamide). Responses were more common in sensitive malignancies – lymphoma, breast cancer, small-cell lung cancer (7/11) – than in less predictably responsive cancers (2/11). Because of the preponderance of sensitive malignancies at cyclophosphamide doses of >6 g/m^2, no conclusions regarding a dose-response effect could be drawn. Nevertheless, the high degree of activity in refractory patients implies that dose intensification can produce responses in resistant tumors, as has previously been noted in murine malignancies [12].

The role of a continuous-infusion schedule of cyclophosphamide administration in anti-neoplastic efficacy will require a comparative study against bolus administration. There are few settings in which single-agent cyclophosphamide would be deemed appropriate therapy in a patient population in which responses are expected. Continuous-infusion cyclophosphamide can be safely and successfully combined with other agents at high doses [9]. To evaluate best the effect of schedule while using the advantage of an increased dose, a combination of cyclophosphamide and other effective drugs given in association with a hematopoietic cytokine to ameliorate the attendant myelosuppression [1] could be tested in a responsive malignancy.

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