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High-dose Cytosine Arabinoside in Multiple Myeloma

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Abstract—*In 14 patients with advanced refractory multiple myeloma, the effect of high-dose cytosine arabinoside (ara-C) administration was evaluated. There was one partial remission among 13 evaluable patients who received 2 g/m² intravenously over 2 hr every 12 hr, for a total of 2–8 g/m² per course, repeated every 3–4 weeks. Myelosuppression constituted the dose-limiting toxicity, causing two treatment-related deaths from infection and bleeding. Prior extensive therapy, a low percentage of cells in S phase and low levels of intracellular ara-CTP accumulation in the bone marrow could explain the resistance of myeloma to this treatment.*

INTRODUCTION

CYTOSINE arabinoside (1-β-D-arabinofuranosylcytosine, ara-C) is a pyrimidine antimetabolite that has established activity against acute leukemia and some antitumor effect in other cancers [1]. It is a cell-cycle-specific agent that exerts its action on cells in S phase through an active product of phosphorylation, ara-CTP. There has been renewed interest in the use of short, intermittent infusion schedules of ara-C to reduce the degree of the dose-limiting myelosuppressive toxicity [2] and to allow the administration of higher doses that might overcome tumor resistance. Encouraged by the results of high-dose ara-C in patients with refractory leukemias and lymphomas [3–5], we initiated a clinical trial in patients with multiple myeloma.

MATERIALS AND METHODS

Patients

Fourteen patients diagnosed to have multiple myeloma were treated with high-dose ara-C after informed consent was obtained. All had had clear-cut evidence of advanced disease refractory to prior chemotherapy that had included combinations of alkylating agents, doxorubicin, vincristine and glucocorticosteroids. All patients had measurable myeloma proteins in serum and/or urine. The abnormal protein was of the IgG type in 11 patients and of the IgA type in three. Five patients had Bence–Jones proteinuria. Most had a performance status ≤2 (Table 1). Patients were required to have a granulocyte count of >1500/μl, a platelet count >100,000/μl and normal liver function tests. Complete blood counts were performed weekly and myeloma protein level was evaluated prior to each course of treatment.

Chemotherapy courses were repeated every 3–4 weeks, depending on bone marrow recovery. Ara-C was given as 2 g/m² intravenously over 2 hr every 12 hr. Based on the schedules of patients with leukemias, our starting dose was 8 g/m²/course. The first two patients treated experienced severe myelosuppression, with one death from bleeding and *Candida* sepsis. This, together with our experience in patients with lymphoma, led us to modify our regimen to a starting dose of 2 g/m²/course, to be increased by increments of 2 g/m² in the subsequent courses if the granulocyte count did not drop bewlow 500/μl with a platelet count >50,000/μl. Response was defined as ≥50% reduction of tumor mass and disappearance of Bence–Jones protein excretion [6].

The concentration of ara-CTP was measured in cells from bone marrow samples taken at the end of the first dose of 2 g/m² of ara-C. Bone marrow aspirated from the posterior iliac crest at the end

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Table 1. High-dose ara-C in multiple myeloma: patients' characteristics

Characteristic	No. of patients
No. of patients entered	14
Median age in yr (range)	60 (48–72)
Male/female	9/5
No. of patients with:	
Performance score 0–2	10
3–4	4
Prior radiation therapy	6
Bone marrow plasmocytosis >10%	14
Bone lytic disease	14
Abnormal elevation of immunoglobulins	14
Bence-Jones proteinuria	5
Extrapulmonary mass	2
Serum blood urea nitrogen >20 mg%	3
Serum creatinine >1.2 mg %	5
Serum calcium >11.5 mg %	0
Tumor mass: high	5
intermediate	9
Median number of prior chemotherapy protocols (range)	4 (2–8)
Median time from diagnosis to therapy in months (range)	42 (6–84)
Median dose of ara-C per course in g/m^2 (range)	4.5 (2–8)

of the ara-C infusion was drawn into a syringe containing heparin as an anticoagulant and transported to the laboratory in an ice bath. After separation of nucleated bone marrow cells by Ficoll-Hypaque density gradient centrifugation procedures, the number of cells and their mean volume was determined for each sample by an electronic particle counter (Coulter Electronics, models ZBI and C-1000 channelyzer). Ara-CTP was separated from cellular nucleotides by high-pressure liquid chromatography exactly as previously described [7]. Nucleotides were detected by u.v. absorbance at 280 nm and quantitated by comparison of the electronically integrated peak areas with predetermined calibration curves. The cellular concentration of ara-CTP was calculated from the knowledge of the amount of ara-CTP detected in an extract of a known number of cells of a determined mean volume. These cells represented all cells collected from the bone marrow aspirate, whether normal or malignant. The ara-CTP cellular concentration was therefore an average value per cell. One marrow aliquot was subjected to flow cytometric analysis of cellular DNA and RNA content as previously described [8]. The percentage of malignant cells was estimated by measuring the percentage of aneuploid cells by acridine orange stain.

Student's *t* test was used to analyze differences in ara-CTP concentrations in relation to myelomatous bone marrow involvement, and percentage of cells in S phase.

In vivo measurements of intracellular accumulation of ara-CTP in bone marrow samples, as well as measurements of the percentage of cells in S phase, were attempted to correlate their relative interaction and possibly predict tumor response.

RESULTS

Thirteen patients were evaluable for response. One patient died from rapidly progressive disease on day 15 of his first course of chemotherapy. All 14 patients were evaluable for toxicity.

Response

The two objective responses were noted at the starting doses of 8 g/m^2 and 4 g/m^2. One patient developed a partial remission manifested by a 50% decrease of his tumor mass. The response lasted 3 months, after which he developed progressive disease. He is still alive 10 months after initiation of high-dose ara-C. A second patient had an objective response with a 35% decrease of the tumor mass. Progressive disease was noted 4 months later, and he is still alive 8 months after high-dose ara-C chemotherapy. The median survival for non-responders was 6 months (range 0–9+ months).

Toxicity

The hematologic toxicity of the regimen was profound (Table 2), with a median lowest granulocyte count of 900/μl (range 0–5000/μl) and a median lowest platelet count of $30 \times 10^3/\mu$l

Table 2. Toxicity of high-dose ara-C in multiple myeloma (14 patients)

Non-hematologic toxicity	% of patients
Nausea and vomiting	64
Drug fever	14
Dizziness	14
Cerebellar ataxia	14
Diarrhea	7

Hematologic toxicity: dose level (g/m² per course)	No. of courses	Median lowest granulocyte count/μl (range)	Median lowest platelet count/μl (range)	Serious toxicity (No. of patients)
2 × 1	5	1.15 (0.2–1.3)	87 (43–206)	—
2 × 2	6	1.2 (0.2–5.0)	52 (25–90)	FUO (1)
2 × 3	7	0.60 (0.0–1.3)	10 (5–28)	*Staph. aureus* sepsis and bleeding + death (1) bleeding (1) FUO (2)
2 × 4	3	0 (0.0–0.1)	10 (10–33)	*Candida* sepsis + bleeding + death (1) FUO and *E. coli* sepsis (1)

(range 5–206 × 10^3/μl). This resulted in two early deaths attributed to chemotherapy complications. One patient died of *Candida* sepsis and bleeding on day 24, with absent granulocytes and a platelet count of 30 × 10^3/μl. The second patient died of *Staphylococcus aureus* sepsis and bleeding on day 15, with a granulocyte count of 100/μl and a platelet count of 10 × 10^3/μl. Furthermore, fever of undetermined origin developed in four patients, *Escherichia coli* sepsis in one and bleeding in three; these were controlled with antibiotics and supportive therapy with platelet and blood transfusions.

Comparison of in vivo *ara-CTP levels and percentage of cells in S phase in patients with multiple myeloma and lymphoma*

Fifteen measurements of ara-CTP and 14 measurements of percentage of cells in S phase were carried out. Most patients showed substantial accumulation of ara-CTP levels in the bone marrow at the end of the first dose, with a median of 68 μM (range 13–270 μM). The two responders had values of 62 and 270 μM, while the median value for non-responders was 64 μM (range 13–215 μM). The median percentage of cells in S phase was 10.5%, with a range of 4–22%.

The results were compared to those obtained in a previous study of high-dose ara-C in patients with lymphoma [5] who received a similar first dose of ara-C (2 g/m²). Twenty ara-CTP measurements and 15 measurements of percentage of cells in S phase were available from lymphoma patients. As shown in Table 3, patients with multiple myeloma had significantly lower accumulation of ara-CTP than lymphoma patients, in addition to a lower percentage of cells in S phase. Contrary to the lymphoma patients, where ara-CTP levels increased with an increasing degree of lymphomatous bone marrow involvement [5], myeloma patients showed a reverse trend: the median ara-CTP level was 141 μM in patients with ≤25% bone marrow involvement compared to a median of 66 μM in patients with >25% bone marrow involvement. The low response and small number of patients studied precluded any correlative analysis between ara-CTP accumulation, or percentage of cells in S phase, and response.

DISCUSSION

The chronic phase of multiple myeloma responds well to melphalan–prednisone or

Table 3. Comparison of ara-CTP levels and percentages of cells in S phase in patients with multiple myeloma and lymphoma

Parameter	Multiple myeloma	Lymphoma	*P* value
Median Ara-CTP levels in μM (range)	68 (13–270)	123 (6–663)	<0.01
Median percentage of cells in S phase (range)	10.5 (4.0–22.0)	22.2 (11.6–39.0)	0.03

similar drug combinations including doxorubicin, vinca alkaloids and nitrosoureas. However, very few agents show any significant clinical activity in myeloma patients resistant to such therapy, stressing the importance of discovering new active regimens.

Experience with conventional doses of ara-C in multiple myeloma is scanty and suggests poor activity of the drug. Based on the demonstrated dose–response correlations in various tumors [9], the delivery of ara-C in a short infusion schedule would reduce the degree of myelosuppression [2] and allow higher doses of the drug to be delivered, hopefully overcoming tumor resistance. Unfortunately, this study using high-dose ara-C resulted in only one partial remission. Such activity was deceiving in view of the better results obtained in patients with refractory leukemias [3, 4] and lymphomas [5].

Moreover, considerable toxicity resulted from such a regimen, with excessive myelosuppression and two treatment-related deaths. Both deaths occurred in the dose ranges where responses were noted. Myelosuppression occurred in spite of the relatively lower doses of ara-C per course used to treat multiple myeloma (2–8 g/m^2) than those used to treat refractory leukemia (12–36 g/m^2). The reason for the better tolerance of leukemic patients to higher doses of chemotherapy remains unknown, but could be attributed to the hematopoietic inhibitory effect exerted by the leukemic cells, which, when abolished by response to chemotherapy, will allow normal bone recovery. In addition, poor tolerance to chemotherapy in this patient population could be related to the prolonged, extensive prior chemotherapy (median duration of 42 months), the use of radiation therapy for palliation of symptomatic bone disease and the myelophthisic marrow invasion by tumor, all of which decrease the bone marrow reserve.

Ara-C is known to exert its antitumor activity primarily on cells in S phase, where the ratio of kinase:deaminase enzymes is highest [10]. The pharmacokinetic and flow cytometry parametric analyses were interesting. Patients with multiple myeloma showed lower concentrations of ara-CTP and lower percentages of cells in S phase in their bone marrows than did lymphoma patients. Interestingly, ara-CTP accumulation decreased with increasing bone marrow involvement by tumor, contrary to patients with lymphoma [5]. Whether ara-CTP levels are related solely to the percentage of cells in S phase or reflect the intrinsic property of different cancer cells to handle ara-C differently remains to be defined. A current study of these parameters in the bone marrows of patients with colon cancer undergoing therapy with high-dose ara-C may help answer this important question. The low response rate (8%) in patients with multiple myeloma compared to lymphoma patients (29%) could be attributed to their lower accumulation of ara-CTP.

In conclusion, high-dose ara-C chemotherapy at the dose schedules studied has a poor activity in patients with refractory multiple myeloma, in addition to excessive toxicity related to bone marrow suppression. A cautious front-line trial in patients with stable multiple myeloma who have not been exposed to excessive bone-marrow-damaging therapy may be considered to test the activity of the drug and its tolerance under similar conditions to those in which alkylating or other agents have shown favorable results. The value of ara-CTP measurements in predicting response and toxicity remains to be defined in larger population studies.

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