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Pilot Study of 5-Azacytidine (5-AZA) and Carboplatin (CBDCA) in Patients With Relapsed/Refractory Leukemia

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5-azacytidine (5-AZA) and carboplatin (CBDCA) are two agents which have demonstrated antileukemic activity in a number of phase I–II trials. Their mechanisms of action and pharmacology related to cell resistance suggested suitability for combination therapy. The aim of this pilot study was to evaluate the effects of this combination in the treatment of patients with relapsed/refractory acute leukemia. A total of 21 patients was enrolled. 5-azacytidine, at doses ranging from 50–150 mg/m²/day, was administered as a 2-hr infusion for 5 consecutive days. On day 3, patients began a 5-day course of CBDCA given as a 24-hr continuous intravenous infusion of 250 mg/m²/day. There were no complete remissions with this regimen. Although there were three partial responses, these were generally of short duration. Nonhematologic toxicities were mild. No correlation was seen between response and serum platinum levels. These results demonstrate that the 5-AZA/CBDCA combination is ineffective therapy for heavily pretreated patients with acute leukemia. © 1996 Wiley-Liss, Inc.

Key words: 5-azacytidine, carboplatin, leukemia

INTRODUCTION

Despite initial success in achieving remission, most patients with acute leukemia relapse and eventually die from the sequelae of the disease. Furthermore, patients who are refractory to standard induction therapy rarely achieve any durable remission with additional chemotherapy alone. Therefore, efforts are continuously underway to identify new agents and combination regimens with improved activity in this disease. Along these lines, carboplatin (CBDCA) and 5-azacytidine (5-AZA) have been combined in a clinical trial for patients with relapsed/refractory acute leukemia based on the antileukemic activity and pharmacology of the individual drugs.

Platinum-containing compounds have gained wide application in the treatment of chemosensitive tumors. They have demonstrated significant therapeutic activity in a large number of metastatic solid tumors [1], and more recently have exhibited activity in hematologic malignancies such as leukemia and lymphoma. Several phase I–II trials using CBDCA as a single agent have reported response rates of 25–45% in patients with acute leukemia [2–5].

5-azacytidine, a pyrimidine analogue similar to cytosine arabinoside (Ara-C), has been used primarily for treatment of hematopoietic malignancies. As salvage therapy in acute leukemia its results have been comparable to other single agents [6–9], but it offers the advantage of a unique mechanism of action [6]. The maximal tolerated dose (MTD) defined by the phase I studies was 150 mg/m²/day for 5 days.

MATERIALS AND METHODS

Patients

Patients >15 years of age with refractory or relapsed acute leukemia and a Karnofsky performance status of >50% were eligible for this study. The study group included individuals with: 1) acute myelogenous leukemia (AML) in first or greater relapse or with primary refractory disease; 2) acute lymphocytic leukemia (ALL) in second or greater relapse or with primary refractory dis-

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ease; and 3) blast crisis of chronic myelogenous leukemia (CML) refractory to at least one course of standard induction chemotherapy. Morphologic confirmation of diagnosis at Memorial Sloan-Kettering was mandatory prior to enrolling in the study. Patients were required to have adequate hepatic (serum bilirubin <1.5 mg/dl) and renal function (creatinine clearance >60 ml/min), no serious hearing impairments, or uncontrolled infections at time of treatment. All patients gave written informed consent prior to participating in the trial, which was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center.

Treatment Regimens

5-azacytidine was administered days 1–5 as a 2-hr intravenous (IV) infusion at doses of 50–150 mg/m^2/day. Dosing was initiated at less than the MTD defined by phase I trials because of concern about possible additive gastrointestinal toxicity. At least 3 patients were treated at each dose level, and doses were escalated by 25 mg/m^2/day as tolerated. CBDCA was given at a dose of 250 mg/m^2/day as a continuous IV infusion, beginning on day 3 and continuing for a total of 5 days. The dose of the CBDCA infusion was based on the results and recommendations of Meyers et al. [3]. The intention of the study was to escalate the dose of 5-azacytidine to its MTD, defined as the dose level before grade 3 or greater toxicity in 2 or more patients was seen. Alternatively, we would escalate the 5-azacytidine to reach its single-agent MTD of 150 mg/m^2. The delayed kinetics of these agents in lowering peripheral blood counts prompted the use of hydroxyurea (HU) when necessary, to control rapidly rising peripheral blast counts or to reduce total white blood cell counts >30,000/mm^3. HU was discontinued at least 24 hr prior to initiating the protocol chemotherapy.

In order to avoid renal toxicity, vigorous IV hydration was used to maintain a urine output of greater than 3 liters/day, and febrile episodes (>100.5°C) were initially treated with ticarcillin/clavulanic acid and aztreonam. Aminoglycosides and amphotericin B were added if the patient became unstable or if fever persisted longer than 48 hr. Ondanestron and decadron were used prophylactically as antiemetics. Decadron was omitted for patients with ALL. Audiograms were obtained at baseline and at completion of the study.

Bone marrow aspirations and biopsies were performed at baseline prior to therapy, 14 days after the start of the treatment, and then at 1–2-week intervals until response was documented. Responses were assessed according to the criteria established by the NCI Workshop [10]. Complete remission was defined as disappearance of all clinical evidence of leukemia for a minimum of 4 weeks with a neutrophil count >1,500 mm^3, platelets >100,000 mm^3, no circulating blasts and a normal marrow differential with maturation in all cell lines, no Auer rods, and <5% blasts. Partial remission was the same as complete remission, except for the presence of 5–25% bone marrow blasts. Failures included patients with resistant leukemia in their bone marrow (>25%) and circulating peripheral blood blasts, and patients who died of complications of aplasia. Clinical evaluation of patients was made daily. Serum chemistries and complete blood counts were performed at least three times a week. Specific organ toxicity was graded according to the DCT/NCI grading system. CBDCA levels were sampled on days 6–7 (96–120 hr into the CBDCA infusion) and assayed using the technique described by Menendez-Botet and Schwartz [11].

TABLE I. Patient Characteristics

Characteristic	Number
Patients enrolled	21
Evaluated for toxicity	20
Evaluated for response	20
Male/female	10/10
Age in years: median (range)	38.5 (21–69)
Karnofsky performance status: median (range)	80% (60–10%)
Type of leukemia	
De novo AML	14
ALL	2
MDS → AML	4
CML → blast crisis	1
Status of disease	
Refractory	8
First relapse	9
Second relapse	3
Prior treatment	
1 course	4
2 courses	8
3 courses	8

RESULTS

Patient Characteristics

Twenty-one patients, whose characteristics are summarized in Table I, were enrolled in the study. The first patient died from a cerebral hemorrhage on the fourth day of chemotherapy, leaving 20 patients evaluble for response. Six of the 20 study patients required treatment with HU for control of a rapidly rising peripheral blast count prior to beginning protocol therapy. All patients enrolled in this study were heavily pretreated: 76% of patients had received ≥two prior therapies and therefore had a poor prognosis for response to other standard therapies. Furthermore, several patients possessed additional unfavorable prognostic characteristics: 2 patients had relapsed after autologous bone marrow transplantation for AML, 1 patient with ALL had previously been treated with radiation and chemotherapy for lymphoma, 1 patient with AML had transformed from a secondary myelodysplastic syndrome, and 1 patient with Ph+ ALL had a number of additional cytogenetic abnormalities. Both ALL patients had relapsed while receiving chemotherapy.

TABLE II. Nonhematologic Toxicity

	Grade			
	1	2	3	4
Stomatitis	4	0	0	0
Nausea/vomiting	1	5	0	0
Diarrhea[a]	0	3	0	1
Neurootoxicity	0	3	0	0
Renal	1	0	0	0
Metabolic[b]	0	0	1	0

[a]Fatal necrotizing hemorrhagic enterocolitis.
[b]Reversible salt-losing nephropathy.

Of the patients treated in first relapse, only one remained disease free >9 months after completing consolidation.

Response

None of the patients treated in this study achieved complete remission (CR). There were 3 patients (treated with 50, 75, and 150 mg/m^2/day of 5-AZA) who obtained a partial remission (PR), making the overall response rate 15%. The median duration of PR was 42 days (range, 35–46 days). One patient who achieved PR on day 59 had normal peripheral blood counts as well as a blast count of <5% in the bone marrow aspirate, but an occasional myeloblast contained an Auer rod. A second patient had only 8% blasts in the bone marrow aspirate on day 33 but subsequently developed isolated leukemia cutis without an increase in marrow myeloblasts on day 68. The time to peripheral count recovery for the 3 patients with PR was long: 53, 28, and 37 days, respectively, for an absolute neutrophil count (ANC) >1,000/mm^3, and 57, 32, and 54 days, respectively, to achieve platelet counts >100,000/mm^3. Fifteen patients had aplastic/hypoplastic bone marrows as a result of therapy, while 5 others manifested primary drug resistance by remaining normocellular/hypercellular.

Toxicity

Therapy with the 5-AZA/CBDCA combination was generally well-tolerated (Table II). The most common extramedullary toxicities were stomatitis (4 patients, grade 1) and gastrointestinal symptoms including nausea/vomiting (1 patient, grade 1; 5 patients, grade 2) and diarrhea (3 patients, grade 2; 1 patient, grade 4). One patient who received 50 mg/m^2/day of 5-AZA, however, developed fever, abdominal pain, and grade 4 diarrhea on day 18. He subsequently expired and was found to have necrotizing and hemorrhagic colitis on autopsy, which was thought to be secondary to the chemotherapy and/or a neutropenic enteropathy.

Three patients experienced transient tinnitus of approximately 1 week duration at the completion of CBDCA. Ten patients underwent the prescribed audiograms. Two patients were symptomatic, having complained of hearing deficits. Both of these patients were found to have significant hearing loss. Two other patients were found to have insignificant high-frequency loss. The 6 remaining patients who were tested, demonstrated no change.

Renal toxicity was mild. Only 1 patient, treated with 150 mg/m^2/day of 5-AZA, developed a salt-wasting nephropathy associated with polyuria and a transient mild increase in creatinine (0.9–1.5 mg/dl). As a consequence of this, however, the patient also developed grade 3 metabolic toxicity comprised of hyponatremia, hypokalemia, hypocalcemia, and hypomagnesemia. These abnormalities resolved with fluid and electrolyte repletion within 14 days. Alopecia was difficult to assess for the entire population because of its presence at baseline in many patients.

The 5-AZA/CBDCA combination did induce a significant degree of myelosuppression. All patients were pancytopenic following therapy and required parenteral antibiotics for neutropenic fever. The time from initiation of chemotherapy until white blood count (WBC) $<1 \times 10^6$/ml was a median of 10 days (8 days after beginning carboplatin). Four patients experienced life-threatening infections (septicemia). In 2 of these patients, causative organisms were identified (*Pseudomonas auerginosa* and *Candida albicans*). Life-threatening hemorrhage occurred in 2 patients. One patient, previously mentioned, developed severe gastrointestinal bleeding in the setting of necrotizing colitis, while a second patient continued to bleed from the site of a central venous catheter which had been removed because of infection. The latter patient was refractory to platelet transfusions and subsequently developed a nonlethal CNS hemorrhage. Of the two deaths which occurred during this study, one was due to hemorrhage alone while the other was due to hemorrhage in the setting of infection.

Carboplatin Levels

Total and free platinum levels were obtained in 18 and 16 patients, respectively (Table III). The median levels of total/free platinum for the entire group were 6.1/3.3 mcg/ml (ranges, 2.0–18.0 [total]/0.3–17.0 [free] mcg/ml). For the 14 patients who achieved hypoplastic marrow, the median levels were 6.8/4.0 mcg/ml (ranges, 2.3–18.0 [total]/0.6–17.0 [free] mcg/ml). The median values for the 4 patients whose bone marrows remained cellular were 4.2/1.9 mcg/ml (ranges, 2.0–5.2 [total]/0.3–2.6 [free] mcg/ml). Platinum levels did not appear to predict for response, since there was a wide range of values in the group achieving PR (11.0/5.8 mcg/ml, 8.2/4.0 mcg/ml, 3.5/1.9 mcg/ml [total/free]).

DISCUSSION

The proposed mechanisms of action of each of these antileukemic agents differ from those of the standard drugs used in the treatment of acute leukemia. Therefore, given the resistance of relapsed/refractory disease to re-

TABLE III. Carboplatin Levels*

Patient	Diagnosis	Creative/Clearance	Total (μg/ml)	Free (μg/ml)	Cellularity (BM d21)	Response
1	AML	100			Aplastic	F
2	AML	66	8.4	3.2	Hypo	F
3	AML	121	11.0	5.8	Hypo	PR
4	AML	101	2.0	0.3	Hyper	F
5	AML	110	5.2	2.4	Hyper	F
6	AML	88	5.0	2.6	Hyper	F
7	AML	78	8.2	4.0	Hypo	PR
8	AML	70	6.8	4.1	Hypo	F
9	ALL	65	2.3	0.6	Hypo	F
10	AML	128	18.0	17.0	Hypo	F
11	MDS-T	79	6.8	4.0	Hypo	F
12	ALL	63	7.1		Hypo	F
13	AML	157	4.3		Hypo	F
14	AML	107	3.5	1.9	Hypo	F
15	AML	71	3.4	1.4	Hyper	F
16	MDS-T	69	6.3	3.3	Hypo	F
17	MDS-T	101	5.9	3.3	Hypo	F
18	MDS-T		3.5	1.9	Hypo	PR
19	CML-T	135			Hyper	F
20	AML	67	8.5	5.4	Hypo	F
Medians		6.1	3.3			

*MDS-T, myelodysplastic syndrome transformed into AML; CML-T, chronic myelogenous leukemia in blast crisis.

peated courses of conventional dose therapy, the rationale for combining 5-AZA and CBDCA is apparent. Furthermore, both drugs offer the additional advantage of having no significant cardiotoxicity. This may be important in a patient population which is heavily pretreated with anthracyclines, such as that studied here.

The study design was based on the biochemical and pharmacologic properties of the drugs. One mechanism of action of platinum-based compounds is the formation of DNA crosslinks via platinum adducts. DNA hypermethylation may protect cells from such adduct formation. In addition, certain cellular enzymes may repair these adducts. Both of these activities have been implicated as mechanisms of platinum resistance [12,13]. The active species of 5-AZA, i.e., the triphosphate, is incorporated into transfer and ribosomal RNA, resulting in direct damage to nucleic acid and indirectly inhibiting the synthesis of proteins such as cellular repair enzymes. Moreover, 5-AZA triphosphate is directly incorporated into and causes hypomethylation of cellular DNA, leading to conformational changes which may alter the DNA interaction with other agents. The sequential use of 5-AZA and CBDCA might be expected to deplete the cells of enzymes capable of repairing platinum-DNA adducts, and to prevent hypermethylation of the DNA.

The CBDCA infusion using 1,250 mg/m^2 (total dose) was based on the recommendation by Meyers et al. [3], which demonstrated a 28.5% response in previously treated patients. The dose of 5-AZA was escalated to a set maximum of 750 mg/m^2 (total) based on reports of antileukemic activity as a single agent at this dose level [9]. Dose escalation beyond this in a combination regimen was felt to be unwarranted.

Although the addition of 5-AZA to CBDCA was well-tolerated in this study, the combination was not effective in inducing remissions in this group of heavily pretreated patients. The meager 15% response rate is comparable to the results of reported studies which have combined CBDCA with other antileukemic agents such as mitoxantrone, daunorubicin, or etoposide [14–16]. The observation that CBDCA combinations produce inferior results compared to those for this drug as a single agent raises concern about the potential utility of such regimens in leukemia therapy. Such results may in part be explained by the heterogeneity of the patient populations treated with the various regimens. Seventy-six percent of patients in the present study had received at least two prior treatment regimens. In contrast, 57% of patients treated with CBDCA alone by Meyers et al., [3] were either untreated or had received minimal therapy prior to first relapse. Furthermore, the potential crossresistance between Ara-C and 5-AZA [7,17] which has been suggested, might also explain the apparent lack of benefit in adding 5-AZA to CBDCA; many patients in the present study had previously failed Ara-C-based salvage regimens.

Pharmacokinetic variability seen among patients, which resulted in differences in plasma drug levels, may also be an important factor in responsiveness to chemo-

therapy. An unexpected finding in this study was the interpatient variation in platinum levels obtained 96–120 hr following initiation of CBDCA infusion. Such levels should have represented a steady state. There appeared to be no correlation between a patient's creatinine clearance (which was required to be >60 ml/min) and platinum levels. It is notable, however, that in the 4 patients who did not achieve hypoplasia, the median total/free platinum levels (4.2/1.9 mcg/ml) were lower compared to those for the other patients (6.8/4.0 mcg/ml). Methods for defining the pathophysiology of this variability of CBDCA serum levels need to be developed in order to use this agent in a more effective manner.

CONCLUSIONS

The challenge in treating patients with refractory/relapsed leukemia lies in the development of strategies to circumvent drug resistance. New antileukemic drugs with novel mechanisms of action and combinations of older drugs may be particularly useful in this regard. CBDCA and 5-AZA have demonstrated activity as single agents, and in this study were administered in combination with an acceptable toxicity profile. The low response rate of this drug schedule, however, argues against its further investigation as a salvage therapy for heavily pretreated patients. In vitro studies may help to define mechanisms responsible for the lack of clinical efficacy of this and other CBDCA-containing regimens.

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