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Effective reinduction therapy for childhood acute nonlymphoid leukemia using simultaneous continuous infusions of teniposide and amsacrine*

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Summary. The combination of teniposide (VM-26) and amsacrine (AMSA) was evaluated in a dose-finding and efficacy study in 58 patients with relapsed or refractory acute leukemia. Both agents were given as simultaneous continuous infusions for 72 h through separate i.v. lines. All patients were evaluable for toxicity and 57 were evaluable for response; only 2 of 20 with acute lymphoblastic leukemia (ALL), acute mixed-lineage leukemia, or chronic myelogenous leukemia in blast crisis achieved a complete remission (CR). More encouraging was a second-remission rate of 43% (13 complete and 3 partial) in the 37 patients with acute nonlymphoid leukemia (ANLL). Responses occurred only in patients who received VM-26 doses of $\geqslant 200$ mg/m^2 per day and AMSA doses of $\geqslant 100$ mg/m^2 per day. Thus, the CR rate for relapsed ANLL patients who received the higher doses of both agents was 40% (13 of 33). All responders had previously received epipodophyllotoxin therapy and 40% had also received AMSA. All but one patient had severe leukopenia ($<2.0\times10^9$ leukocytes/l) and thrombocytopenia ($<50.0\times10^9$ platelets/l) as a result of therapy. Severe mucositis (grade 3 or 4) was the dose-limiting toxicity. Our results indicate that VM-26 plus AMSA, given by continuous infusion, is effective in the treatment of ANLL. Further phase II studies should consider using VM-26 at 200 mg/m^2 per day and AMSA at 100 mg/m^2 per day, but the best administration schedule remains unclear.

Introduction

In an attempt to identify useful new drug combinations for acute leukemia, we evaluated the drug pair teniposide (VM-26) and amsacrine (AMSA). The exact efficacy of VM-26 as a single agent in leukemia has not been established, but continuously infused VM-26 has produced antileukemic effects in patients with acute leukemia [5, 14]. In an attempt to obtain complete remissions (CRs), we combined VM-26 with AMSA, a drug that has shown substantial activity against acute leukemia [2, 6, 9, 10]. Since both VM-26 and AMSA appear to exert their effect through topoisomerase II-mediated interactions with DNA [15], we reasoned that simultaneous drug exposure might result in more pronounced antileukemic effects. The rational for selecting a simultaneous continuous-infusion schedule was to expose leukemic cells continuously to both drugs during the period of DNA synthesis.

Material and methods

Patients. All 58 patients with treatment-resistant leukemia enrolled in this trial between July 1984 and November 1987 are included in this report. In all, 51 patients were consecutively entered at St. Jude Children's Research Hospital, 6 were entered at the Dana Farber Cancer Institute, and 1 was entered at the University of California, Davis. The protocol was approved by each institution's clinical trials review committee; informed consent for therapy was appropriately obtained from patients or their parents. Before each cycle of therapy, the following values were recorded for all patients: potassium, >3.5 mEq/l; creatinine, <2 mg/dl; blood urea nitrogen (BUN), <40 mg/dl; bilirubin, <2.5 mg/dl; serum glutanic oxaloacetic transaminase (SGOT) or serum glutanic pyruvic transminase (SGPT), <200 IU/l; and normal echocardiogram ejection fractions.

Treatment. VM-26 and AMSA were given simultaneously through separate i.v. lines for 72 h. AMSA was given through a central venous catheter to prevent phlebitis and ECGs were monitored during the infusion. A loading dose of VM-26 was used to rapidly achieve a steady-state plasma concentration [14]. The dose escalations used in the present trial are presented in Table 1. If a bone marrow aspiration or biopsy carried out 7 days after the beginning of the infusions demonstrated >5% residual leukemic cells, additional therapy at the same dose level was started within 36 h. Only two induction cycles were given when the VM-26 dose was increased to 250 mg/m^2 per day and AMSA was given at doses of $\geqslant 100$ mg/m^2 per day; however, patients treated at lower doses could receive up to three induction cycles. Patients achieving CRs were eligible to receive up to three cycles of maintenance therapy, each of which was identical to induction.

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Table 1. Schedule of dose escalation and CR at each dose

Dose escalation	VM-26 (mg/m²)		AMSA (mg/m²)	CR/number treated			
	Loading dose[a]	Infusion (per day)	Infusion (per day)	ANLL	ALL	Other[b]	Total
1	13	150	75	0/1	0/2	0	0/3
2	13	150	100	0/3	0	0/1	0/4
3	18	200	100	5/7	1/7	0/2	6/16
4	45	250	100	2/4	0/2	0	2/6
5	45	250	125	2/4	0/1	0	2/5
6[c]	45	250	150	4/18	0/3	1/2	5/23

[a] For dose levels 1–3 the loading dose was given over 30 min, whereas for dose levels 4–6 it was given over 1 h
[b] Acute mixed-lineage leukemia ($n = 4$), chronic myelocytic leukemia in blast crisis ($n = 1$)
[c] One patient treated at dose level 6 was not evaluable for response

Table 2. Characteristics of all 58 patients enrolled

	ANLL	ALL	Other[a]	Total
Number of patients	38	15	5	58
Male:female	25:13	8:7	2:3	35:23
Age (years)[b]	8.5 (0.5–27)	11.0 (1.5–23.5)	12.2 (7.3–13.9)	8.2 (0.5–27)
WBC ($\times 10^9$/l)[b]	3.6 (0.5–252.0)	4.2 (0.8–186.0)	7.4 (3.9–94.3)	3.9 (0.5–252.0)
Peripheral blasts (%)[b]	10 (0–97)	22 (0–96)	23 (6–92)	11 (0–97)
Marrow blasts (%)[b]	57 (10–100)	76 (25–100)	80 (34–100)	70 (6–100)
Treatment history:				
Induction failure	8	2	1	11
Refractory relapse[c]	5	5	1	11
Hematologic relapse (1)	19	1	1	21
Hematologic relapse (2 or more)	6	7	2	15
Relapse on therapy	25	15	4	51
Number of drugs received	9 (4–13)	9 (6–10)	7 (6–8)	9 (4–13)
Previous VM-26 or VP-16	37	15	4	56
Previous AMSA	22	0	2	24

[a] Acute mixed-lineage leukemia ($n = 4$), chronic myelocytic leukemia in blast crisis ($n = 1$)
[b] Median (range); other values are numbers of patients
[c] Patients had failed at least one previous reinduction attempt before entering this trial

Definition of response and toxicity. A CR was defined as a marrow aspirate with restored hematopoiesis, <5% blast cells, no definite leukemic blasts, normal blood counts, and a normal performance status for >1 month. A partial remission (PR) was defined as an absence of peripheral blasts, <25% marrow blasts, and recovery from all toxicity. Toxicity was graded according to criteria of the Pediatric Oncology Group.

Results

The clinical characteristics and treatment histories of all 58 patients are summarized in Table 2. One child with chronic myelocytic leukemia in blast crisis died during drug-induced marrow aplasia, and another with ANLL refused the second cycle of reinduction therapy; both children are included in the analysis as failures of reinduction therapy. One patient with acute progranulocytic leukemia (FAB M3) with a documented t(15; 17) was enrolled in this trial. She was treated with daunorubicin and cytarabine followed by etoposide and azacytidine as initial induction therapy but failed to enter remission. She then received 6-mercaptopurine, vincristine, methotrexate, and prednisone (POMP). A marrow examination carried out 6 weeks after the start of POMP demonstrated increased numbers of blasts (5%) and dysmorphic progranulocytes (17%). The patient was therefore started on VM-26 and AMSA; however, cytogenetic marrow studies carried out prior to this therapy, which were reported later, did not confirm the presence of a t(15;17). She received two cycles of VM-26 and AMSA, and a marrow examination done 6 weeks after the start of therapy revealed clearing of all dysplastic myeloid cells, a blast count of 10%, and a normal karyotype. Since this patient's karyotype was normal before and after VM-26 and AMSA treatment, she was considered not evaluable for response.

In the 37 children with ANLL who were evaluable for response, treatment with VM-26 and AMSA induced CRs in 13 and PRs in 3, for an overall response rate of 43%. All CRs occurred in the 33 patients with ANLL who were treated at the higher doses of VM-26 (≥ 200 mg/m² per day) and AMSA (≥ 100 mg/m² per day), but the small number of ANLL patients ($n = 4$) treated at lower doses prohibited statistical analysis (Table 1). Only three patients achieved a CR after one (72 h) cycle of therapy; the other

Table 3. Clinical characteristics of patients achieving CR

Patient	FAB type	Relapse on or off therapy	Previous			Induction cycles (*n*)	Total dose (mg/m^2)		Duration of CR (months)
			VM-26	VP-16	AMSA		VM-26	AMSA	
1	M2	Off	No	Yes	No	1	600	300	5
2	M4	On	No	Yes	Yes	1	750	375	2
3	M2	On	Yes	Yes	Yes	2	1200	600	5
4	M2	On	No	Yes	Yes	2	1200	600	6
5	M1	Off	No	Yes	No	2	1200	600	6
6	M1	On	Yes	Yes	No	2	1200	600	4
7	M5	On	No	Yes	Yes	2	1500	600	2
8	M1	On	No	Yes	Yes	2	1500	750	3
9	M1	On	Yes	Yes	Yes	2	1500	900	5
10	M2	On	No	Yes	Yes	2	1500	900	BMT
11	M1	Off	No	Yes	Yes	2	1500	900	4
12	M4	On	No	Yes	Yes	2	1500	900	BMT
13	M2	On	Yes	Yes	No	3	1800	900	1
14	ALL	On	Yes	No	No	2	1200	600	2
15	Mixed	On	No	Yes	No	1	750	450	BMT

BMT, removed from study for bone marrow transplantation

Table 4. Toxicity at each dose escalation[a]

Dose escalation	Patients treated	Number of patients with grade 3 or 4 toxicity[b]					
		WBC $<2\times10^9$/l	Platelets $<50\times10^9$/l	Mucositis	Nausea, vomiting	Diarrhea	Hepato-toxicity
1	3	3	3	1	0	1	0
2	4	4	4	3	1	1	0
3	16	16	16	7	0	2	1
4	6	6	5	4	0	1	1
5	5	5	5	3	0	0	0
6	24	23	24	10	2	1	1
Total	58	57 (98%)	57 (98%)	28 (48%)	3 (5%)	6 (10%)	3 (5%)

[a] Please see Table 1 for exact doses given
[b] According to Pediatric Oncology Group criteria

ten who achieved a CR required two or three cycles of induction therapy (Table 3).

Eight ANLL patients in their first hematologic relapse and five in their second hematologic relapse achieved a CR. CRs were obtained in 10 of 25 ANLL patients who relapsed during maintenance therapy. Maintenance therapy in these responding patients had previously included vincristine, doxorubicin, cytoxan, cytarabine, 6-thioguanine, and etoposide or high-dose cytarabine, doxorubicin, etoposide, and azacytidine [8]. All but 1 of the 37 evaluable children with ANLL who were enrolled in the present study had received prior treatment with epipodophyllotoxins (VM-26 or VP-16), yet CRs were obtained in 13. All responding patients had received epipodophyllotoxins within 1 year (range, 2 weeks to 1 year; median, 6 weeks) of being enrolled in the present trial. Likewise, of 22 patients with ANLL who received AMSA therapy between 6 weeks and 1 year (median, 3 months) prior to being enrolled in this trial, 9 achieved CRs. It is impossible to determine whether patients were refractory to VM-26 or AMSA, since most patients were receiving multiagent maintenance therapy including etoposide and AMSA, but at lower doses, when they relapsed. Patients with FAB M1, M2, or M3 leukemia appeared more likely to achieve a CR (10 of 23) than those with the M4 or M5 subtype (3 of 11), but this was not statistically significant (Table 3). None of the three patients with megakaryocytic leukemia (FAB M7) achieved a CR.

This drug combination was not as effective in the 15 patients with relapsed or refractory ALL; only 1 achieved a CR and 1, a PR. One of the four patients with mixed-lineage leukemia achieved a CR [12]. The clinical characteristics and prior therapy of all patients who achieved a CR are included in Table 3. The child with chronic myelogenous leukemia in refractory blast crisis developed marrow aplasia without obvious residual leukemia but died of fungal sepsis.

Toxicity. In all, 98% of the patients required platelet transfusions and 98% developed severe leukopenia ($<2.0\times10^9$ leukocytes/l) (Table 4); all patients had neutropenia ($<0.5\times10^9$ neutrophils/l). When all patients who underwent a CR are considered, hematopoietic recovery (neutrophils, $>0.5\times10^9$/l; platelets, $>50\times10^9$/l) occurred at a median of 28 days (range, 15–41 days). Time to hematopoietic recovery was shorter in the three patients who re-

quired only one induction cycle (median, 22 days; range, 20–24 days). No clear relationship appeared to exist between the dose level given and the duration of myelosuppression. Almost all patients (97%) were hospitalized due to fever and 67% received amphotericin B. Ten patients (17%) had documented bacterial infections, and seven (12%) had documented fungal infections.

Mucositis occurred in 88% of the patients and was severe (grade 3 or 4) in 48% (Table 4); it was clinically worse (more often grade 4) at the highest dose levels and was the dose-limiting toxicity. Hepatoxicity was not a major problem, being transient in all patients. Only four children developed grade 3 or 4 hepatotoxicity, but one had a documented cytomegalovirus (CMV) infection with apparent CMV hepatitis. None of the patients experienced cardiac dysrhythmia or showed clinical evidence of cardiac failure. No pulmonary, neurologic (central or peripheral), genitourinary, or pancreatic toxicity occurred.

Only 6 of the 15 patients who achieved a CR were treated with maintenance chemotherapy. Maintenance therapy resulted in grade 3 or 4 hematopoietic toxicity after each cycle and mild to moderate gastrointestinal toxicity (grade 1 or 2) after most cycles. Remissions were of short duration in all patients, the median length being 4 months (range, 1.3–6 months) (Table 3). Three patients underwent bone marrow transplantation after achieving a CR on this protocol; all are still alive, with no evidence of recurrent disease. The six remaining patients who achieved a CR refused maintenance therapy, since the toxicity was significant and the effectiveness of maintenance therapy in ANLL patients in their second CR is doubtful.

Discussion

Reinduction rates for patients with ANLL have ranged from 20% to 70% [2, 4, 7, 9–11, 16–18]. Our results demonstrate that simultaneous continuous infusion of VM-26 and AMSA is effective reinduction treatment for ANLL patients. The overall response rate (CR + PR) was 43% despite prior epipodophyllotoxin therapy in almost all patients (98%) and previous AMSA therapy in most patients (60%). Patients with FAB M1, M2, or M3 leukemia appeared to be more likely to respond than those with the M4 or M5 subtype, but this was not statistically significant. The majority of patients who underwent a CR (10 of 13; 77%) required more than one 3-day cycle of induction therapy to achieve remission, suggesting that 72-h continuous infusion therapy is too short. Unfortunately, the small numbers of patients enrolled at each dose prevented meaningful statistical analysis of dose-response or dose-toxicity relationships.

Experience with VM-26 as a single agent in the treatment of relapsed ANLL is limited, but the drug may be particularly effective in acute monocytic leukemia [5, 8, 13, 17]. AMSA has been adequately studied as a single agent in ANLL, producing CR rates of up to 28% at a total dose of ≥ 450 mg/m^2 [2, 9, 10, 17]. The CR rate of 40% in the present trial exceeds the results obtained with either VM-26 or AMSA alone, suggesting improved clinical results with the combination. Combination chemotherapy for relapsed ANLL that includes AMSA has yielded very promising results, with CR rates approaching 70% when AMSA is combined with high-dose cytarabine [7, 18].

Recent studies similar to ours have been reported [1, 11, 16]. Abrams et al. [1] used daily short infusions of VP-16 and AMSA (both at 100 mg/m^2 per day for 5 days); their CR rate was only 19% and their patients experienced severe gastrointestinal toxicity. Tschopp et al. [16], who treated relapsed ANLL patients with AMSA (120 mg/m^2 per day for 5 days) and continuous infusion VP-16 (80 mg/m^2 per day for 5 days), obtained results similar to ours and induced second CRs in 56% of their patients, most of whom had myeloid subtypes of ANLL (FAB M1, M2, and M3).

Essentially all of our patients (98%) had previously received epipodophyllotoxin therapy, and most (60%) had also received AMSA; therefore, they might be expected to respond less well to retreatment with the same agents. Since the epipodophyllotoxins have been most effective in cases with the M4 or M5 leukemia subtypes [8, 13], the higher response rate obtained in the present study in patients with the myeloid leukemic subtypes suggests that AMSA was the most active agent.

We would suggest that doses of 200 mg/m^2 VM-26 and 100 mg/m^2 AMSA be used in subsequent phase II studies, since these doses appeared to be equally effective but less toxic than the higher doses we gave. However, the small number of patients enrolled at each dose escalation precludes a clear dose-response relationship. We are unsure as to what contribution each drug or the continuous infusions might have made to our results in patients with ANLL. Our CR rate in recurrent ANLL was similar but not superior to that obtained in a number of other studies, but the hematopoietic and gastrointestinal toxicity in this study was severe. Furthermore, simultaneous continuous infusion of both drugs was complicated and required hospitalization. Additionally, since most patients required two cycles of induction therapy to achieve a CR, the duration of the infusions (72 h) was too short. Since the total dose of AMSA we gave over 10 days was very high (up to 900 mg/m^2) and was tolerable even when combined with high-dose VM-26, rather than continuing the present trial, we feel that further phase I studies of continuous infusion AMSA using a different dose or infusion duration are indicated.

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