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Evaluation of Aniline Mustard in Patients with Multiple Myeloma[1]

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SUMMARY

Aniline mustard, an alkylating agent effective in plasma cell tumors in BALB/c mice, was used in a prospective randomized study of 44 patients with multiple myeloma. None of the patients had received prior chemotherapy, and only three had had radiation. The patients were randomized between two regimens, one consisting of aniline mustard, 1 mg/kg, and the other of aniline mustard, 1 mg/kg, plus prednisone, 1.2 mg/kg in decreasing dosage. Thirty-three patients were treated for 30 days or more and were eligible for evaluation of response. Nine patients responded in two or more parameters. Myeloma progressed during the study in all but three patients. Twenty-two of the 31 responses occurred in patients who received prednisone in addition to aniline mustard. Approximately two-thirds of the responses occurred in the first 60 days of therapy. Toxicity consisted mainly of leukopenia. Infections occurred in 10 patients, and a skin rash was noted in 2 others. Aniline mustard possesses some activity in multiple myeloma but is inferior to melphalan.

INTRODUCTION

The effect of L-phenylalanine mustard (melphalan, Alkeran) has been well established in the treatment of multiple myeloma (1, 2, 6). However, response to this agent is not optimal, and thus there is a need for new drugs in the treatment of this disease.

Aniline mustard [*N*-di-2-chloroethylaniline (CB-1074)] was reported to produce a greater effect against ADJ/PC5 plasma cell tumors in BALB/c mice than did either cyclophosphamide or melphalan (9, 10). It was postulated that aniline mustard is hydroxylated in the liver to form a highly toxic *p*-hydroxyaniline mustard which was then detoxified in the liver by conversion to the glucuronide or sulfate moiety. The high glucuronidase or sulfatase activity of the tumor resulted in the selective release of *p*-hydroxyaniline mustard. Indeed, these plasma cell tumors had increased levels of β-glucuronidase when compared with the liver, the spleen, or other tumors (3). However, increased levels of β-glucuronidase have not been demonstrated in human myeloma.

Little information on the clinical effect of aniline mustard has been published. Healy (4) reported that of 6 patients with myeloma 2 showed significant decrease in paraproteins within 2 or 3 weeks when given aniline mustard, but leukopenia with slow recovery was a problem. Young *et al.* (11) found that 1 of 9 patients with myeloma who were given aniline mustard became stabilized.

MATERIALS AND METHODS

All patients fulfilled 1 of these 2 criteria for the diagnosis of multiple myeloma. (*a*) The bone marrow contained abnormal atypical plasma cells in excess of 40%, together with a clinical picture compatible with myeloma, or (*b*) the bone marrow contained atypical or abnormal plasma cells in excess of 5%, or there were soft tissue masses histologically compatible with the diagnosis of multiple myeloma, plus at least 1 of the following: M spike demonstrated in serum by electrophoresis on filter paper or cellulose acetate; M spike (globulin) demonstrated in urine by filter paper or cellulose acetate electrophoresis; or roentgenographic evidence of characteristic osteolytic lesions. Other diseases characterized by plasmacytosis in the bone marrow such as metastatic carcinoma, collagen diseases, and cirrhosis were excluded. Patients acceptable for this study had an estimated survival of at least 2 months, an initial leukocyte count of 3,500/cu mm or greater, an initial platelet count of 90,000/cu mm or more, a blood urea nitrogen level of less than 30 mg/100 ml, a serum calcium level of less than 12 mg/100 ml, and no significant infection. All eligible patients had received no alkylating agent, no prior corticosteroid within 10 days of the study, and no recent radiation therapy.

Patients who fulfilled these criteria were randomly allocated to 1 of 2 treatment regimens. Patients in 1 treatment regimen received aniline mustard in a single daily p.o. dose of 1 mg/kg; while those in the other regimen received aniline mustard in the same dosage plus prednisone, 1.2 mg/kg daily p.o. for 2 weeks, 0.8 mg/kg daily for 2 weeks, 0.4 mg/kg daily for 2 weeks, and then 0.2 mg/kg daily p.o. for 4 weeks.

Prednisone was added to 1 therapeutic regimen because it increases the effectiveness of melphalan in the therapy of

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multiple myeloma (1). Aniline mustard was given to previously untreated patients because response to a new alkylating agent is often suboptimal when given to patients previously treated with alkylating agents.

The initial evaluation included history, physical examination, and determinations of hemoglobin or hematocrit value, leukocyte count (total and differential), platelet count, reticulocyte count, blood urea nitrogen or creatinine, blood uric acid, and serum calcium as well as serum protein electrophoresis, urinalysis, examination for Bence Jones proteinuria, total protein determination and electrophoresis of a 24-hr urine specimen, studies of bone marrow aspirates, and a roentgenographic skeletal survey. During therapy, determinations of hemoglobin or hematocrit values and leukocyte and platelet counts were repeated weekly; serum electrophoresis, urine electrophoresis, and determinations of blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, and alkaline phosphatase and bone marrow aspirations were advised every 4 weeks. All initial studies were repeated when the study of each patient was terminated. The performance of each patient was arbitrarily rated from 0 through 4: 0, normal activity; 1, mild symptoms but ambulatory; 2, moderate to severe symptoms but in bed less than 50% of the waking time; 3, severe symptoms (in bed more than 50% of the waking time); and 4, completely bedridden.

Response was evaluated by the application of the following definitions of several parameters of the disease: (*a*) hemoglobin, an increase of 2 g or more per 100 ml, persisting at least 2 weeks in the absence of blood transfusions in patients whose initial hemoglobin concentration was 11 g or less per 100 ml, or an increase in the hematocrit value of 5.5% (initial value, 35% or less) under the same circumstances; (*b*) serum globulin, a decrease of 2.5 g or more per 100 ml, from an initial value of more than 4.5 g or a return to normal values or a disappearance of the globulin spike; (*c*) urinary globulin, a decrease of at least 50% from an initial value of 1.0 g or more per 24 hr; (*d*) plasma or myeloma cells, a decrease of 200 per 1000 total marrow cells; (*e*) recalcification of bone lesions in the absence of new lesions developing during the study; (*f*) improvement of performance by 2 grades; and (*g*) decrease of pain by 2 grades (pain was graded on the basis of 0 through 4). Patients had to be treated for 30 days or more to be included in evaluation of response.

Aniline mustard was available in 25-, 50-, and 100-mg tablets and was administered p.o. The dosage of aniline mustard was continued daily and altered during therapy, depending on blood counts (Table 1).

Table 1
Dosage schedule for aniline mustard in treatment of multiple myeloma

Leukocytes/ cu mm	Platelets/ cu mm	Dose (% of standard dose)[a]
>5,000	>100,000	100
4,000–4,999		75
3,000–3,999	75,000–100,000	50
2,000–2,999		25
<2,000	<75,000	0

[a] Standard dose, 1.0 mg/kg body wt/day.

OUR SERIES

Forty-four patients were entered in the study. Records of 2 patients were not received and, therefore, not evaluated, and 1 patient failed to meet the criteria for entry. Of the remaining 41 patients, 8 others were not included in this present evaluation because they were treated for less than 30 days. Three of the 8 patients moved away and could not be followed; 3 died of infection, but their leukopenia was only moderate and chemotherapy probably did not have a significant role in their deaths; 1 patient died of cardiac arrest; and 1 died less than 1 month after dismissal from the hospital, but no additional information could be obtained. Six of the 8 patients had received less than 1,200 mg of aniline mustard, and all 8 had received less than 2,100 mg of the drug.

Of the remaining 33 patients (17 men and 16 women) in the series, 17 received aniline mustard plus prednisone, whereas 16 received aniline mustard alone (Table 2). None of the patients had received prior chemotherapy, and only 3 had been given radiation therapy. Two-thirds of the group had the onset of symptoms 6 months or less before treatment was begun, and 31 of the 33 evaluable patients were given aniline mustard within 3 months of the diagnosis of myeloma. Thus, chemotherapy was given relatively early in the course of the disease.

RESULTS

Two of the 22 patients who were anemic (less than 13.0 g, hematocrit 39%, for males and 12.0 g, hematocrit 36%, for females) at onset of therapy had significant increases in levels of hematocrit. Both of these patients with significant increases in hematocrit were on the prednisone-aniline mustard regimen. In 17 originally anemic patients, the anemia worsened. Nine of 11 patients with a normal initial hemoglobin level developed anemia during the study.

The leukocyte count decreased to 3,000 or less per cu mm in 26 patients, but only 3 had leukocyte counts of less than 1,000. The lowest value of the leukocyte count was not seen at any particular point in the period of treatment. Platelet counts of less than 100,000/cu mm occurred in 12 patients, the count being less than 50,000 in only 2 of them. Bleeding from thrombocytopenia did not occur. Maximal thrombocytopenia did not occur at any characteristic time in the period of therapy.

The serum globulin concentration decreased by at least 2.5 g/100 ml in 6 patients, and the M spike disappeared in 3 others. Eight of these 9 patients were on the prednisone-aniline mustard regimen. Of the remaining 24 patients, 19 had no significant decrease in globulin levels, 4 had normal globulin levels initially, and for 1 there were no follow-up data. The urinary M spike decreased by more than 50% in 3 of the 7 patients in whom it was greater than 1 g/24 hr and adequately evaluated; 2 of the 3 patients were on the prednisone-aniline mustard regimen.

A significant decrease in plasma cells (200 per 1000 nucleated cells) occurred in 6 patients. One of these patients had a decrease of more than 50%. Two-thirds of the group

Table 2
Treatment of 33 patients with multiple myeloma

Case	Sex	Age (yr)	Hemoglobin (g/100 ml)[a]	Serum globulin (g/100 ml)[a]	Plasma cells in marrow (%)[a]	No. of parameters abnormal at onset	Improvement		Bone lesions	Status
							No. of parameters	Duration (days)		
Aniline mustard plus prednisone										
1	F	82	9.3	6.8	55.5	6	0	0	Pos.[b]	Alive
2	M	74	14.4	5.3	22	4	0	0	Pos.	Alive
3	M	58	13.2	4.9	99	5	0	0	Pos.	Alive
4	F	72	9.2	8.8, 6.1	52, 4	3	2	209, 186	Neg.	Alive
5	M	59	14.6	3.1	18	3	0	0	Pos.	Alive
6	F	68	12.8	3.3	49	2	1	>84	Neg.	Dead
7	M	74	9.3	4.1, 2.2[c]	7	4	2	56, >43	Neg.	Dead
8	F	60	12.6	7.9, 5.3	16	5	1	44	Pos.	Alive
9	F	71	12.8	4.7, 2.8[c]	20	5	3	>300, 231, >236	Pos.	Dead
10	F	61	30.5, 36[d]	7.6	71, 32	6	4	23, (?), 70, 25	Pos.	Dead
11	M	70	8.9	6.3	40	4	0	0	Pos.	Dead
12	F	69	10.3	3.8	6	4	0	0	Pos.	Dead
13	F	31	23.5, 31[d]	7.1	100	6	1	49	Pos.	Alive
14	F	50	9.8	10.5, 7.4	37	6	1	(?)	Pos.	Alive
15	M	73	14.0	2.8, 2.5[c]	21	4	2	251, 259	Pos.	Alive
16	M	42	13.4	7.2, 4.5	28,2	3	2	>61, 47	Pos.	Alive
17	M	50	9.1	7.1, 4.4	40, 10	4	3	>742, (?), 516	Neg.	Alive
Aniline mustard										
18	M	62	14.7	4.3	27	5	1	194	Pos.	Alive
19	F	66	9.3	10.5, 6.8	15	6	2	497, >534	Pos.	Alive
20	M	51	13.5	6.5	40	4	1	>14	Neg.	Alive
21	F	57	9.9	6.7	56, 24	5	1	(?)	Pos.	Dead
22	F	33	8.4	7.6	30	4	1	>63	Neg.	Alive
23	F	57	9.9	4.7	95, 29	6	1	>58	Pos.	Alive
24	M	61	13.6	2.3	7.6	4	2	231, 217	Pos.	Alive
25	M	46	9.4	10.6	95	6	0	0	Pos.	Dead
26	M	55	12.5	7.0	27	6	0	0	Pos.	Alive
27	F	70	9.5	6.2	69	4	0	0	Neg.	Dead
28	F	50	11.2	1.6	100	4	0	0	Pos.	Dead
29	F	51	11.4	4.9	20	4	0	0	Pos.	Alive
30	M	54	11.5	6.9	21	4	0	0	Pos.	Alive
31	M	59	12.5	2.6	44	7	0	0	Pos.	Alive
32	M	66	9.2	6.9	70	6	0	0	Pos.	Dead
33	M	53	9.3	1.9	36	4	0	0	Pos.	Dead

[a] When 2 values are given, 2nd values signify improvement in that parameter.
[b] Pos., positive; Neg., negative.
[c] Serum M spike disappeared.
[d] Hematocrit (%).

with a significant decrease in plasma cells were on the prednisone-aniline mustard regimen.

Healing of bone lesions was not found in any patient. Nineteen had progression of bone lesions consisting of new lytic lesions, compression fracture, or pathological fracture; 9 of these patients were on the prednisone-aniline mustard regimen. Hypercalcemia developed in 2 patients during therapy, and azotemia occurred in 1 patient.

A pain decrease of 2 or more grades was noted in 7 patients (3 on the prednisone-aniline mustard regimen), and a decrease of 1 grade was noted in 4 other patients. The severity of pain was unchanged in 8 patients and was worse in 7; 6 patients were free of pain during the study. The performance of 10 of 26 evaluable patients who had limitation of activity initially improved to some degree; it improved by 2 grades or more for 4 patients (3 on prednisone-aniline mustard regimen). The performance of 12 patients decreased during the study. Pain and performance improved significantly in 1 other patient (Case 28), but the improvement was thought to be from previous radiation therapy.

Among the 31 parameters that responded, the duration of response was greater than 6 months for 14 and less than 2 months for 9. Improvement in pain and performance accounted for 7 of the 14 parameters with more than 6 months of improvement. The improvement was still present in 10 parameters at the time of the current evaluation. Approximately two-thirds of the parameters that improved reflected improvement within 2 months of the onset of therapy.

Total dosage of aniline mustard was less than 2 g for 3

patients, and none of these derived any benefit. Seven of 16 patients who received aniline mustard obtained benefit only in 1 or more parameters, whereas 11 of 17 improved in the group that received aniline mustard and prednisone (Table 3). Five of 8 parameters that were initially abnormal improved in patients who received more than 10 g of aniline mustard. Twenty-two of the 31 isolated responses occurred in patients who had received the prednisone-aniline mustard combination (Table 4).

All patients are off the study because of no response to therapy or a response followed by progression of disease, as manifested by increased lytic bone lesions, increased fractures, increased serum or urine protein, decreased hemoglobin, increased plasma cells in the marrow, increased pain, or decreased performance, except for 1 patient in whom a skin rash prevented continued treatment with aniline mustard. Even in the patients with continuing improvement, therapy had to be discontinued because of progression of other significant parameters. Of the 33 patients, 21 are still alive.

Toxicity consisted mainly of leukopenia and thrombocytopenia. Infections occurred in 10 patients during the study. Leukopenia of less than 2000/cu mm was present in 6 of these patients and may have contributed to infection. Eight of the 13 episodes of infection occurred in patients on the prednisone-aniline mustard regimen. Skin rash occurred in 2 patients.

DISCUSSION

The treatment of patients with multiple myeloma is a difficult challenge and requires varied therapeutic regimens. Radiation therapy is helpful for localized lesions, but in most instances the disease is too widespread for radiation to be of clinical value as the only mode of therapy. Urethan is of little value in the therapy of multiple myeloma (5). L-Phenylalanine mustard (melphalan, L-sarcolysin, L-PAM, Alkeran) has produced clinical benefit in several studies (1, 2, 6). Cyclophosphamide, another alkylating agent, also produces improvement in myeloma (7).

The response to chemotherapeutic agents is difficult to monitor because the tumor mass cannot be directly measured. The reduction of serum and urine M protein probably correlates directly with the number of plasma cells in the patient. This problem is being studied by Sullivan and Salmon

Table 3

Dosage of aniline mustard in treatment of multiple myeloma

	Aniline mustard				Aniline mustard plus prednisone			
	Patients		Parameters		Patients		Parameters	
Total dose (mg)	No.	Responding	Abnormal[a]	Responding	No.	Responding	Abnormal[a]	Responding
<2,000	2	0	8	0	1	0	6	0
2,000–4,000	5	3	25	3	6	5	27	10
4,000–6,000	4	0	20	0	5	2	23	4
6,000–10,000	5	4	23	6	3	2	8	3
>10,000	0	0	0	0	2	2	8	5
Total	16	7	76	9	17	11	72	22

[a] This does not include 3 parameters not evaluated and 2 that responded to radiation therapy.

Table 4

Response of parameters to therapy of multiple myeloma

	No. of parameters			
	Aniline mustard		Aniline mustard plus prednisone	
Parmeter	Abnormal[a]	Responding	Abnormal[a]	Responding
Hemoglobin	13	0	9	2
Serum protein	13	1	15	8
Urine protein	5	1	2	2
Pain (2 or more grades)	9	4	9	3
Performance (2 or more grades)	7	1	8	3
Marrow	16	2	16	4
Bone lesions	13	0	13	0
Total	76	9	72	22

[a] This does not include 3 parameters not evaluated and 2 that responded to radiation therapy.

(8), who have measured the amount of immunoglobulins secreted by the plasma cells.

The use of prednisone apparently contributed to the responses in this study. Only 9 of 76 abnormal parameters responded to aniline mustard alone, whereas 22 of 72 abnormal parameters responded to aniline mustard plus prednisone. Of the 31 parameter responses, 11 measured pain and performance, which are subjective responses. Thus, only 4 of 60 abnormal parameters responded to aniline mustard alone if the subjective responses of pain and performance are excluded (Table 4).

The rationale for the use of aniline mustard is based on studies of mice with plasmacytomas containing increased amounts of β-glucuronidase. The β-glucuronidase of the tumor interferes with the detoxification of *p*-hydroxyaniline mustard (the active component of aniline mustard). However, increased levels of β-glucuronidase have not been demonstrated in human myeloma, and this might account for the inferior results found in the present study. Mouse plasmacytomas may not be a satisfactory predictive model for selection of chemotherapeutic agents in human myeloma.

Although aniline mustard was more efficacious than either melphalan or cyclophosphamide in plasma cell tumors in mice, it appears inferior to these agents in humans.

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