Plasma cell myeloma: response of melphalan-resistant patients to high-dose intermittent cyclophosphamide

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Summary: High-dose intermittent cyclophosphamide was used in the treatment of 19 patients with plasma cell myeloma who had demonstrated resistance to melphalan. Good objective improvement was found in six patients and partial responses in five. Patients have survived from three to more than 36 months (estimated median 21 months) after starting cyclophosphamide, and 11 are still alive. The important implications of the lack of cross-resistance between these two alkylating agents are discussed in terms of the mechanisms of cellular resistance to alkylating agents.

Résumé: *Le myélome à plasmocytes: réaction des malades melphalano-résistants à une forte posologie intermittente de cyclophosphamide*

Chez 19 malades souffrant de plasmocytome et qui s'étaient révélés résistants au melphalan, les auteurs ont utilisé une forte posologie intermittente de cyclophosphamide. Une sensible amélioration objective a été noté chez six malades et une réaction partielle chez cinq autres. Les malades ainsi traités ont survécu durant une période variant de trois mois à plus de 36 mois (moyenne estimée 21 mois) après le début du traitement et 11 sont encore vivants. Les auteurs étudient les implications importantes de l'absence de résistance croisée entre ces deux agents alkylants, notamment en ce qui concerne les mécanismes de la résistance cellulaire aux alkylants.

Both melphalan and cyclophosphamide are effective in the treatment of plasma cell myeloma. The administration of melphalan by a variety of dosage schedules[1-3] has been reported to produce significant tumour regression in 30 to 50% of patients and to prolong survival. The daily administration of cyclophosphamide appears to be equally effective in causing tumour regression and survival prolongation.[4-6] The treatment of patients with a combination of melphalan and prednisone[1] and melphalan, prednisone and procarbazine[7] has increased the proportion of responding patients, but has not resulted in improved survival as compared with the survival of patients treated with melphalan alone.

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Alexanian *et al*[8] have shown that the median survival from the onset of melphalan therapy is 41 months for patients who have a good response, as compared to nine months for unresponsive patients. By subtracting the time spent in remission from the survival of the responding patients, it has been shown that the improvement in survival can be completely accounted for by the time spent in remission.

Better methods are required for the induction and maintenance of remissions in patients with plasma cell myeloma. More thought should be given to the use of another alkylating agent in patients who fail to respond or who relapse while on melphalan therapy. The purpose of the present study was to test the effectiveness of high-dose intermittent cyclophosphamide therapy in patients with demonstrated resistance to melphalan. We found that many of these patients obtain good objective responses.

Patient selection and methods

At The Princess Margaret Hospital patients with plasma cell myeloma are treated initially with intermittent melphalan and prednisone. Since March 1969 patients who demonstrate resistance to this form of therapy have been changed to high-dose intermittent cyclophosphamide. The clinical features of the 19 patients treated in this way are shown in Table I. The median interval from diagnosis to the start of cyclophosphamide therapy was 18 months, with a range of eight to 74 months. These patients had been treated with melphalan for a median of 16 months, with a range of two to 57 months. When cyclophosphamide was started 17 patients showed evidence of disease progression, namely increasing bone pain, increasing lytic skeletal lesions, enlarging plasmacytomas and a rising myeloma protein (M-protein) concentration in the serum or excretion in the urine. Four of these patients were relapsing after a good response to melphalan, and in five patients the presence of thrombocytopenia (platelet count less than 50,000/c.mm.) was an added reason for changing from melphalan to cyclophosphamide. Case no. 6 had a fall in platelet

count to 10,000/c. mm. after the first course of melphalan; cyclophosphamide was substituted since it was believed that melphalan was not adequate therapy. Cyclophosphamide was given to two patients who had failed to improve following treatment with adequate doses of melphalan for six and 57 months; the disease was relatively stable in both of these patients (cases 5 and 13).

The diagnosis of plasma cell myeloma was based on the demonstration of bone marrow plasmacytosis, with plasma cells accounting for more than 10% of the nucleated marrow cells. Roentgenograms showing skeletal lesions were followed serially after starting therapy. Electrophoresis was done on cellulose acetate, using the Beckman Model R-100 microzone system. Serum M-proteins were calculated (g./100 ml.) and measured serially after starting therapy. The types of heavy and light chains of the M-protein were determined by immunoelectrophoresis. A qualitative test for urine protein was performed with sulfosalicylic acid on all subjects. When positive the presence of light-chain (Bence Jones) M-protein was established by electrophoresis and/or immunoelectrophoresis of a concentrated urine specimen. Urine protein excretion (g./24 hrs.) was measured serially after electrophoresis had established that virtually all of the urine protein represented Bence Jones protein. Hemoglobin, total leukocyte and differential counts, platelet counts, blood urea nitrogen and serum calcium measurements were performed on all subjects and repeated at appropriate intervals.

Melphalan therapy was initiated with doses of 0.25 mg./kg./day for four days, repeated at six-week intervals. When prednisone was used it was given in doses of 100 to 200 mg./day for four days with melphalan or cyclophosphamide. Cyclophosphamide was given either intravenously in a single dose of 1.0 g./m.^{2} or orally in doses of 0.25 g./m.^{2}/day for four days; these doses were repeated at three-week intervals. When cyclophosphamide was given orally patients were instructed to take the drug in the morning and to drink at least 3000 ml. of fluid during the day. These patients were also advised to empty their bladders as frequently as possible to avoid the development of a chemical cystitis. The doses of melphalan and cyclophosphamide were increased or decreased until the doses which depressed the leukocyte nadir to between 1000 and 2000/c. mm. were determined; these doses were then repeated. Patients who developed hypercalcemia were treated with intravenous fluids and prednisone until the serum calcium returned to normal. Painful skeletal lesions were treated with radiation therapy.

The following criteria were used in the evaluation of the response to therapy:

Table I

Melphalan-resistant patients treated with high-dose intermittent cyclophosphamide

Case no., sex, age	M-Protein	Melphalan		Cyclophosphamide			Present Status
		Schedule (Mo.)	Response	Reason for change	Schedule (Mo.)	Response	
1, F, 52	G/k	M, daily (12) M + P (4)	? Progression	Progression + ↓ platelets	Cy + P (9)	Progression	Dead
2, M, 48	G/λ	Cy, daily (24) M (40)	? Progression	Progression	Cy + P (22)	Progression	Dead
3, M, 57	G/k	M + P (10)	Progression	Progression	Cy + P (3)	Progression	Dead
4, M, 50	G/k	M + P (12)	Progression	Progression	Cy + P (8)	Progression	Dead
5, M, 54	G/k	M (29) M + P (28)	No response	No response	Cy + P (8)	Progression	Alive
6, F, 47	G/k	M (2)	Progression	Progression + ↓ platelets	Cy + P (24)	Partial	Alive
7, M, 69	G/k	M + P (6)	Progression	Progression	Cy + P (4)	Partial, improving	Alive
8, M, 59	G/k	M, daily (18)	Partial	Relapse	Cy (iv) + P (3)	Good	Alive
9, M, 48	G/k	M (5) M + P (2)	Progression	Progression + ↓ platelets	Cy (iv) + P (4)	Good	Death, Ca. lung
10, M. 62	G/k	M (36) M + P (5)	Good	Relapse	Cy (iv) + P (5)	Good, then relapse	Death
11, M, 56	G/λ	M + P (23)	Good	Relapse	Cy (iv) + P (16)	Good	Alive
12, M, 66	G/k + A/k	M + P (23)	Good	Relapse	Cy + P (8)	Good	Alive
13, F, 70	G/λ + M/λ	M + P (6)	No response	No response	Cy (iv) + P (35)	Good	Alive
14, F, 60	A/λ	M + P (16)	Progression	Progression + ↓ platelets	Cy (iv) + P (12)	Progression	Dead
15, F, 56	A/λ	M, daily + P (47)	?	Progression	Cy + P (4)	Progression	Alive
16, M, 56	A/k	M (11)	Good	Relapse	Cy (11) Cy + P (10)	Progression	Dead
17, F, 57	k	M + P (11)	Progression	Progression	Cy + P (6) Cy (11)	Partial	Alive
18, M, 55	k	M, daily (17)	?	Progression + ↓ platelets	Cy + P (9)	Partial	Alive
19, M, 41	None	M + P (16)	Progression	Progression	Cy + P (21)	Partial	Alive

1. *Good response.* This required a change in at least one of the following direct manifestations[9] of the plasma cell tumour: (a) a decrease in serum M-protein concentration or urine M-protein excretion to less than 50% of the pretreatment value; (b) more than a 50% regression in the product of the two largest diameters of plasmacytomas; or (c) radiographic evidence of skeletal healing. These patients also showed symptomatic improvement, with an increase in performance status[10] of 20% or more, maintained a hemoglobin concentration of 10 g./100 ml. or more without transfusions and showed a rise in serum albumin if this was low before starting treatment.

2. *Partial response.* Patients who were symptomatically improved, with an increase of 20% or more in performance status and some improvement in a direct manifestation of plasma cell myeloma (but less than the improvement required for a good response), were considered to have a partial response.

3. *No response.* No change in performance status and no change in any of the direct manifestations of the tumour.

4. *Progression.* These patients developed increasing symptoms, reduced performance status, a rising serum M-protein concentration or urine M-protein excretion, new lytic skeletal lesions or the appearance and progressive growth of plasmacytomas.

The responses of four patients to their initial treatment before referral to The Princess Margaret Hospital could not be evaluated. Indirect evidence suggests that cases 15 and 18 had a good response to this initial treatment.

Table II

Response of melphalan-resistant myeloma patients to high-dose intermittent cyclophosphamide

Response	No. of patients	Survival
Good	6	3+, 5, 7, 10+, 15+, 36+
Partial	5	3+, 13+, 20+, 20+, 24+
No improvement	1	24+
Progression	7	4, 5+, 8, 11, 14, 19, 24
Total	19	

Survival, in months after initiation of cyclophosphamide therapy, is indicated; + beside a number signifies that the patient is still alive.

Results

The results of treating melphalan-resistant myeloma patients with high-dose intermittent cyclophosphamide are summarized in Table II. A good response occurred in six patients; four of these patients are still alive and the good response has been maintained for three to 36 months. Of the two good responders who have died one showed complete disappearance of the serum and urine M-protein, but he succumbed at five months to a squamous-cell carcinoma of the lung; the response in the other patient was brief, and he died with a rapidly progressive myeloma seven months after starting cyclophosphamide.

An example of a good response to melphalan and cyclophosphamide, illustrated by the changes in the serum M-protein concentration, is shown in Fig. 1.

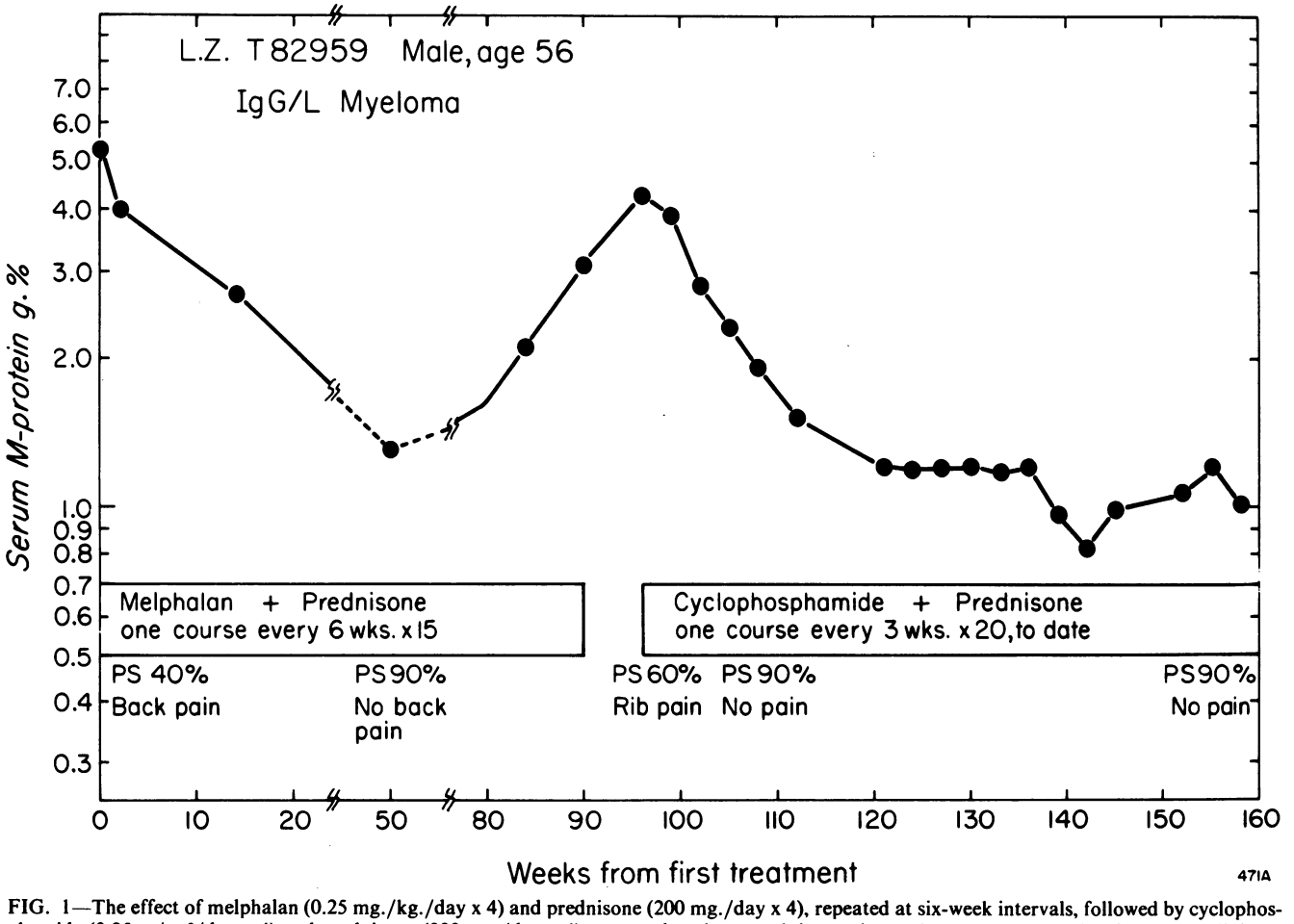

FIG. 1—The effect of melphalan (0.25 mg./kg./day x 4) and prednisone (200 mg./day x 4), repeated at six-week intervals, followed by cyclophosphamide (0.25 g./m.²/day x 4) and prednisone (200 mg./day x 4), repeated at three-week intervals, on the serum M-protein of case 11. All drugs were administered orally. PS = performance status, Karnofsky scale.[10]

All of the five patients classified as showing a partial response are still alive after three to 24 months of cyclophosphamide therapy. One of these patients has been followed for only three months and his serum M-protein is still falling; it is possible that this patient will eventually achieve a good response.

One patient with stable myeloma failed to respond to both melphalan and cyclophosphamide therapy, but his disease continues to be stable and he is alive 24 months after starting cyclophosphamide. The myeloma continued to progress in seven patients after they started on cyclophosphamide and six of these have now died.

The majority of these patients have been long survivors, as one would expect, for only those patients who survived an adequate trial of melphalan were included in the study. Since more than half of the patients are still alive it is not possible to calculate survival accurately, but it is estimated by the life-table method[11] that the median survival from diagnosis will be about 47 months (range 11 to 101 months), and the median survival from the start of cyclophosphamide therapy will be 21 months.

Discussion

For many years chemotherapists have assumed that a tumour which is resistant to one alkylating agent will also be resistant to other alkylating agents. This assumption is probably not true. Ogawa, Bergsagel and McCulloch[12] have shown that the mouse plasma-cell tumour MOPC 460D is relatively resistant to melphalan and 1,3-bis(2-chloroethyl)-1-nitrosourea but is very sensitive to cyclophosphamide. The present clinical trial of high-dose intermittent cyclophosphamide for the treatment of melphalan-resistant myeloma patients has shown that good objective responses can be achieved in some of these patients, and it seems likely that the survival of the six patients who obtained a good response and the five patients with a partial response will be prolonged by this therapy. It is clear that the resistance of plasma cell tumours to melphalan therapy does not mean that the tumour will also be resistant to cyclophosphamide.

Several mechanisms have been suggested to explain the development of resistance to an alkylating agent. The possible mechanisms include the following: an increased cellular concentration of protective agents (e.g. thio groups) which spare critical target sites from lethal injury by alkylation;[13, 14] the presence of enzyme(s) which either circumvent a specific metabolic block or enhance the capacity for repair;[15, 16] and alteration of membrane permeability to the drug.[17-20] Many factors appear to be involved in the development of resistance to an alkylating agent,[21] but it has been clearly established that resistance to nitrogen mustard is characterized by reduced uptake of the drug. Goldenberg *et al*[20] have shown that the uptake of nitrogen mustard by L5178Y lymphoblasts occurs by a carrier mechanism and is an active process; nitrogen mustard appears to be carried into these cells by the same transport-carrier mechanism used for choline.[22] Goldenberg *et al*[20] have also shown that chlorambucil, melphalan and cyclophosphamide do not inhibit the uptake of nitrogen mustard by L5178Y lymphoblasts. This observation suggests that the transport mechanism for nitrogen mustard differs from that used by other alkylating agents, and it may well be that there is a different transport-carrier for each alkylating agent. Since a reduction in the uptake of an alkylating agent by tumour cells appears to be an important factor in the development of resistance to these drugs, and there appears to be more than one transport mechanism for carrying alkylating agents into cells, it seems likely that other examples of the absence of cross-resistance between alkylating agents will be discovered.

These observations have important implications for cancer chemotherapy, for patients who develop resistance to either melphalan or cyclophosphamide should have a trial of the other alkylating agent. Lin and Bruce[23] have shown that BCNU and cyclophosphamide act synergistically if both drugs are administered concurrently in the treatment of the KHT sarcoma; melphalan and cyclophosphamide are also synergistic in the treatment of this tumour.[24] A well-designed clinical trial will be required to determine whether it is better to treat myeloma patients with one agent until resistance develops before the other agent is started, or whether two or more alkylating agents should be given alternately or concurrently.

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Retrospect

The study of symptoms

Every art tends to be destroyed by the machinery it creates; the tools of the trade may become more important than the trade itself. There is no lack of examples and perhaps our legal friends would agree that it often seems as if the machinery and procedure of the law have come to mean more than the basic principles of justice. Medicine is no exception and it seems worth while considering whether we are not in danger of making more of the machinery than of the art itself. It is a commonplace to dwell on the increasing complexity of medicine and to point out the difficulty of keeping up with all the advances. For the majority of us who have dates of the last century on our diplomas there is difficulty in understanding some of the work published to-day. The youngsters speak a language which we have not learned and perhaps, for our comfort be it said, this has been true of many previous generations....

It is a constant struggle for teachers to keep their students from making more of the machinery than of the art, and there is a certain type of student who, when asked, for example, the diagnostic points of some thoracic disease, answers first that an x-ray examination should be made. This may be necessary and may be the last court of appeal, but that is no reason why the usual methods of examination should not be carried through first.

One unfortunate result of this habit of mind is that the individual's own powers of observation and reflection atrophy through disuse. Trusting to a mechanical or laboratory method brings its punishment — the individual can do no other. Perhaps the statement that the powers have atrophied through disuse is not always correct — they may never have been present. If this is the case it may be difficult to do much, but for the majority the matter is largely one of training and here the teachers of medicine have a heavy responsibility....

My feeling is that one very important thing for the profession to-day is to make the effort to get back to fundamentals. After all the essential things do not change. Proper methods and thoroughness are as important to-day as they were fifty years ago and as they will be fifty years in the future. The great majority of diagnoses have to depend principally on the proper use of our own faculties. Much of what comes to us as raw material to be fashioned into a correct diagnosis is in the form of the patient's account of his symptoms. This brings one naturally to the problem of obtaining these symptoms. It is very evident that there can be no complete study of symptoms until they have been obtained, so that if we expect to use them properly we must first obtain them to work on. This has to do largely with the question of the history.

How can we ensure the best stock of material to work with? This is not intended as a discourse on history taking but some words on it may not be amiss. How many of us take a better history to-day than we did a year ago? This should always be our aim. Three things stand out, patience, thoroughness and system. With this goes a training in examination and cross-examination as thorough as any lawyer should have. — Thomas McCrae: Can Med Assoc J *12: 610, 1922.*