Cancer Chemother Pharmacol (1986) 16: 165–169

Cancer Chemotherapy and Pharmacology

Treatment of advanced neuroblastoma with high-dose melphalan and autologous bone marrow transplantation*

O. Hartmann[1], C. Kalifa[1], E. Benhamou[1], C. Patte[1], F. Flamant[1], C. Jullien[1], F. Beaujean[2] and J. Lemerle[1]

[1] Department of Pediatrics, Institut Gustave-Roussy, 39 à 53, rue Camille Desmoulins, F-94805 Villejuif Cedex, France
[2] Centre Départemental de Transfusion Sanguine, Avenue Delattre de Tassigny, F-94000 Creteil, France

Summary. Fifteen children with advanced neuroblastoma according to Evans' classification (1 with stage III and 14 with stage IV) were treated with high-dose melphalan (HDM) followed by autologous bone marrow transplantation. Before HDM, all patients had been extensively treated with multimodality therapy for a median duration of 9 months. At the time of HDM, seven children were in partial remission (PR) with measurable residual tumor and 8 were in complete remission (CR) or good partial remission (GPR). No reduction in measurable tumor size was observed in any of the PR patients. However, when HDM was used as consolidation therapy (CR and GPR patients) survival appeared encouraging, since five of eight patients are alive with no evidence of disease at (NED) 29^+ to 54^+ months after HDM. Tolerance of this high-dose chemotherapy was satisfactory; gastrointestinal toxicity appeared to be the most important limiting factor. These results suggest that chemotherapy including high-dose melphalan is promising when used as consolidation therapy in patients who have already attained CR with conventional therapies.

Introduction

The prognosis of metastatic neuroblastoma treated with conventional therapy has remained particularly poor in past years, both in our experience [8] and in that of others [4, 6, 11, 18]. The results for stage III disease (Evans' classification) [3] are no better when the tumor is nonresectable [8, 18]. In 1979, McElwain et al [16] published encouraging results in advanced neuroblastoma treated with high-dose melphalan (HDM) and autologous bone marrow transplantation (ABMT). We therefore designed a study with the same protocol as that described by these authors for patients with advanced neuroblastoma. We report here the results obtained with this regimen and its tolerance in 15 patients with poor-prognosis neuroblastoma.

Patients and methods

Patients. Fifteen patients with advanced neuroblastoma were treated with high-dose melphalan; their characteristics are shown in Table 1. The median age at diagnosis was 2 years (range: 6 months to 13 years), and the male: female ratio was 13:2. According to Evans' classification [3] there were 14 stage IV and 1 nonresectable stage III cases and no stage IV s. Details of primaries and extension are also shown in Table 1.

Before treatment with high-dose melphalan all patients had been extensively treated. As shown in Table 2, they had received three to five different drugs for a median period of 9 months (range 5–17). Surgical excision of the primary was attempted in 9/15 patients; it resulted in six complete excisions, two incomplete excisions, and one biopsy of a tumor which remained nonresectable. For the six remaining patients surgery was not performed, either because of nonresectability (patients 2, 3, 5, 6, and 15) or because the site of the primary was not known (patient 14).

As a result of primary therapies (Table 2), seven patients had cytologically normal bone marrow but still had measurable disease in the primary site and regional lymph nodes (patients 2, 3, and 4) or in metastatic sites (patients 1, 6 and 7) or both (patient 5). Disease status was stable for patients 1–5 and progressive for patients 6 and 7. The results of the primary therapies in this group (group I) were termed partial response (PR). In group II, four patients presented with complete disappearance of all metastatic signs and a small macroscopic residual primary tumor ($\leqslant 5\%$ of the initial size) assessed by surgery and/or CT scan (patients 8, 13, and 15) or microscopic residual primary tumor after surgical excision (patient 11). Their response to primary therapy was termed good partial response (GPR). The four remaining patients of this group were in complete remission (CR) with no detectable disease. Two were in first CR (patients 10 and 12), and two were in second CR after a first bone marrow relapse (patients 9 and 14).

Methods. Melphalan was administered as an IV bolus through a central venous line with hyperhydration ($3 l/m^2$ per day). The dose used was 140 mg/m^2 for 11 patients (2–8, 11–13, and 15) and 180 mg/m^2 for four (1, 9, 10, and 14). Furosemide (1 mg/kg IV) was used systematically for the first 12 of these 15 patients and for the others when it was necessary to maintain adequate diuresis.

The technique of bone marrow harvesting has been described elsewhere [21]. Samples of bone marrow harvest were cytologically screened for residual involvement. For four patients (patients 2, 9, 12, and 13) bone marrow was cryopreserved in DMSO as previously described [9]. For

* This study was presented in part at the workshop on high-dose chemotherapy with autologous bone marrow transplant. (Cancer Therapy evaluation program, NCI, Bethesda, March 1984)

Offprint requests to: O. Hartmann

Table 1. Patients characteristics at diagnosis

Patient no.	Age/sex	Stage	Site of primary	Sites of metastases		
				Bone	Marrow	Other
Group I						
1	8/12/ M	IV	A	+	+	DLN, L, SC
2	4 M	IV	A	+	+	P
3	13 M	IV	A	−	−	DLN
4	7/12/ M	III	A	−	−	−
5	2 M	IV	A	+	+	L
6	4 M	IV	A	+	+	−
7	4 M	IV	A	+	+	−
Group II						
8	14/12/ F	IV	T	+	−	−
9	13/12/ M	IV	A	+	+	−
10	6/12/ M	IV	A	+	+	−
11	2 M	IV	A	+	+	−
12	3 M	IV	A	+	+	−
13	11/12/ M	IV	A	+	+	−
14	9 M	IV	Unknown	+	+	−
15	6 F	IV	A	−	+	DLN

A, abdomen; T, thorax; DLN, distant lymph nodes; L, liver; SC, subcutaneous site(s); P, pleura

Table 2. Primary therapy before high-dose melphalan

Patient no.	Chemotherapy		Surgery	Status before HDM
	Drugs	Duration (months)		
Group I				
1	CVA	12	Complete excision	PR
2	CVAD	7	No surgery	PR
3	CVA	9	No surgery	PR
4	CVAD	14	Biopsy	PR
5	CVAD	7	No surgery	PR
6	CVAD	8	No surgery	PR
7	CVAD	5	Complete excision	PR
Group II				
8	CVAD, VM	12	Incomplete excision	GPR
9	CVAD	8	Complete excision	CR
10	CVA	9	Complete excision	CR
11	CVAD	6	Complete excision	GPR
12	CVA	9	Complete excision	CR
13	CVAD	6	Incomplete excision	GPR
14	CVA, VM, P	10	No surgery	CR
15	CVAD	17	No surgery	GPR

C, cyclophosphamide; V, vincristine; A, adriamycin; D, DTIC; VM, VM_{26}; P, cisplatinum; PR, partial response; GPR, good partial response; CR, complete response

the remaining patients bone marrow was not cryopreserved, but was stored at 4°C for 8 or 24 h before reinfusion.

All patients were treated in simple reverse isolation barrier conditions. During aplasia, they received irradiated blood products and parenteral nutrition when needed.

No systematic gut decontamination was used, and when temperature rose above 38°C patients received broad-spectrum antibiotics.

Complete and differential blood counts were performed three times a week from the beginning of high-dose chemotherapy until normal hematological values were reached.

Evaluation of response. To measure the response to primary therapies before HDM and the response to HDM, patients were extensively reassessed on two occasions, before bone marrow harvest and after hematological recovery following bone marrow transplantation.

Primary tumor and regional lymph nodes were measured during surgical exploration and/or CT scan. Metastases were measured by clinical examination, bone radio-

isotope scan, and hepatic ultrasonography. Bone marrow was considered as cleared after cytological examination of ten samples taken from ten different sites and histological study of one trephine biopsy. Urinary excretion of the catecholamine metabolites VMA, HVA, and dopamine was systematically studied.

Evaluation of toxicity. The severity of gut toxicity (vomiting, mucositis and diarrhea) was established as already described elsewhere [15].

For evaluation of infectious complications, the criteria were the duration of fever with body temperature over 38°C and the occurrence of sepsis and/or documented infections.

Results

Tumor response

Group I patients (1–7) presented with measurable bulky disease prior to high-dose melphalan. One single temporary shrinkage of the primary was observed (patient 2). No measurable effect of HDM was observed among the six remaining patients.

Patients 8–15 (group II) presented with no measurable tumor prior to HDM and were not evaluable for tumor response.

Histological examination of surgical specimens obtained after high-dose melphalan showed fibrotic tissue in one patient (15) and residual poorly differentiated neuroblastic tissue in all the others (2, 3, 4, 8 and 13). Details are given in Table 3.

Survival

One of the 15 patients (no. 6), died early of toxicity, and 14 were evaluable for duration of response. Eight of these are still alive: two had stable disease at 33+ and 49+ months after ABMT; two relapsed 17 and 44 months after ABMT; one is alive, with disease, at 50^+ months; and the other died 31 months after ABMT. Five were free of disease at 29^+, 33^+, 39^+, 50^+, and 54^+ months after ABMT. These five patients had minimal residual disease or none at all before HDM, and four of them received further local therapy after bone marrow transplantation. Ten of these 14 evaluable patients received additional therapy after ABMT. Details are given in Table 3.

The six patients who received additional chemotherapy after HDM are not truly evaluable for duration of survival after high-dose melphalan *alone.*

Toxicity

All patients experienced profound myelosupression. The nadir value for granulocytes was $< 0.1 \times 10^9$/l, and that for platelets $< 20 \times 10^9$/l.

The median duration of granulopenia $< 0.5 \times 10^9$/l in the 14 evaluable patients was 14 days (range 6–24). Leukopenia with $\leqslant 1 \times 10^9$/l lasted for a median of 10 days (range 6–20), and the median duration of thrombocytopenia $\leqslant 50 \times 10^9$/l was 20 days (range 13–200).

Gut toxicity was essentially marked by diarrhea. Nausea and vomiting were absent or of mild intensity. Only 1 patient experienced moderate mucositis, but 5/15 had moderate or severe diarrhea.

The incidence of infection was relatively low. Two patients had no fever during the aplastic phase, while the median duration of fever in the others was 7 days (range 0–12). Ten patients had fever of unknown origin and three had documented septicemia. In one of these cases septicemia was fatal. One patient died of subacute viral infection 5 months after HDM.

Table 3. High-dose melphalan – response – survival

Patient n°	Measurable disease prior to HDM	Measurable effects of HDM	Additional therapy after HDM	Outcome	
				Status	Months
1	DLN, L	0	RX, C	Alive static	33+
2	Primary	Temporary shrinkage	S, C	Died of disease	12
3	Primary, DLN	0	S, RX	Relapse 44 months after HDM; alive with disease	50+
4	Primary, RLN	0	S, RX, C	Alive, static	49+
5	Primary, RLN, L	0	C	Died of disease	5.5
6	Bones	NE	0	Died of toxicity	12 days
7	Bones	0	C	Died of disease	8
8	Minimal residual primary	0	S, RX, C	Alive, NED	50+
9	0	0	0	Alive, NED	33+
10	0	0	RX	Alive, NED	29+
11	Minimal residual primary	0	0	Relapse 9 months after HDM (BM); died of disease	16
12	0	0	0	Died of toxicity	5
13	Minimal residual primary	0	S, RX	Alive, NED	39+
14	0	0	0	Relapse 17 months after HDM (BM); died of disease	31
15	Minimal residual primary	0	S	Alive, NED	54+

DLN, distant lymph nodes; L, liver; RX, radiotherapy; C, conventional chemotherapy; S, surgery; RLN, regional lymph nodes; NE, not evaluable

One patient with acute nonlymphocytic leukemia (no. 13) was observed 2 years after HDM; this patient is still alive. No other late effect was observed in the long-term survivors.

Discussion

For our patients with measurable residual disease no significant tumor response was observed. McElwain et al [16] Pritchard et al. [21], and Graham-Pole et al. [7] reported significant shrinkage of the disease with the same therapy. Nevertheless, in their studies [16, 21] most of the patients received fewer drugs during primary conventional chemotherapy, and for a shorter period of time. In the last study quoted [7] patients were in progressive relapse at the time of HDM, and the dose and schedule of HDM administration were different. Our sample comprised a selection of children with particularly resistant disease, extensively treated for a median of 9 months. These differences may be the best explanation of our less impressive results.

One of our patients with bulky residual disease prior to HDM (no. 3) remained free of disease for 44 months and finally relapsed at the primary site.

Three of the remaining evaluable patients with residual bulky disease prior to HDM (no. 2, 5, and 7) died of disease progression within 1 year despite further therapy; the other two nos. 1 and 4) are still alive with stable disease, follow-up now amounting to more than 3 years after HDM. These two patients received conventional chemotherapy after HDM and are not truly evaluable for duration of response after HDM *alone*.

Group II patients ($n=8$), who were given HDM either in CR or in GPR without measurable disease, are not evaluable for antitumor response, but are evaluable for survival duration. As shown in Table 3, only one of these patients (no. 8) received conventional chemotherapy after HDM and is not truly evaluable for survival after HDM *alone*. The seven remaining patients in this group had no further therapy for their metastases. The treatments administered after HDM were focused exclusively on the primary. In this group, two patients relapsed 9 and 17 months after HDM, but five of the eight are alive and free of disease 29^+ to 54^+ months after HDM. With this small number of patients, no statistical comparison can be significantly demonstrated. However, in another study [8], of stage IV neuroblastoma patients older than 1 year at diagnosis who were treated in the same institution with the same conventional therapies without HDM, 19 of 61 patients (31%) were in CR for more than 6 months and only 2/19 (10%) are long-term survivors. With conventional therapy the median disease-free survival observed in CR metastatic neuroblastoma is usually short, lasting from 15 to 18 months [8, 18], and the long-term disease-free survival rate never exceeds 10% [4, 8, 22].

In the present series, four of the five long-term survivors were 6–14 months of age at diagnosis (8, 9, 10, and 13). However, three of them had multiple bone metastases with clinically bulky bone masses, especially on the skull (8, 10, and 13) and one (no. 9) was in second CR after a first bone marrow relapse.

The prognosis of stage IV neuroblastoma is known to be better in children under 1 year of age [12], but in our hands, with the same conventional therapy only two of seven stage IV patients under 1 year at diagnosis are long-term survivors. Since patients were not randomly assigned to receive high-dose melphalan, no statistical demonstration can be made. However, with five of eight long-term survivors among CR or GPR patients, the results of HDM in this group are encouraging. The results of the ENSG randomized trial comparing survival of stage IV neuroblastoma patients with and without HDM used as consolidation therapy will be the only way of determining whether this therapy is an important factor in the improved survival rates being obtained.

The difference in survival observed in our two groups of patients is close to that obtained for other malignancies treated by high-dose regimens with BMT. Studies of leukemias treated by high-dose chemo-radiotherapy and allogeneic bone marrow transplantation have demonstrated much better results in patients treated in CR rather than in progressive disease [5, 20]. Our study of children's malignant lymphoma showed the same results [9].

In the present study, intensive supportive care and expert nursing were necessary to limit the adverse effects of HDM. They permitted satisfactory tolerance of this regimen in these young children, who had previously been extensively treated. Major myelotoxocity was regularly observed but was usually of short duration. The role of ABMT in shortening the duration of the aplastic phase has already been demonstrated [10, 17]. As in other studies [2, 7, 13, 14, 19] extrahematological toxicity was essentially manifested as diarrhea. Gut toxicity appears to be the major limiting factor for dose escalation of HDM.

We conclude that as in other studies [7], HDM with ABMT support is tolerable in children with advanced neuroblastoma. In patients previously treated extensively no measurable effects were demonstrable in those with bulky residual disease, but in those with minimal residual disease the long-term disease-free survival rate is encouraging and further investigation is warranted. A new study of advanced neuroblastoma treated in CR by HDM combined with other drugs and followed by purged ABMT is now in progress.

References

1. Beaujean F, Hartmann O, Le Forestier C, et al (1984) Autologous cryopreserved bone marrow in 40 patients: In vitro studies and clinical results. Biomed Pharmacother 38: 348–352
2. Cornbleet MA, McElwain TJ, Kumar PT, et al (1983) Treatment of advanced malignant melanoma with high-dose melphalan and autologous bone marrow transplantation. Br J Cancer 48: 329–334
3. Evans AE, d'Angio GL Randolph J (1971) A proposed staging for children with neuroblastoma. Cancer 27: 374–378
4. Finklestein JZ, Klemperer MR, Evans AE, et al (1979) Multiagent chemotherapy for children with metastatic neuroblastoma. A report from the children's cancer study group. Med Pediatr Oncol 6: 179–188
5. Gale RP (1980) Clinical trials of bone marrow transplantation in leukemia. In: Gale RP, Fox CF (eds) Biology of bone marrow transplantation. Academic, New York, p 11
6. Gasparini M, Bellani FF, Musumeci R, et al (1974) Response and survival of patients with metastatic neuroblastoma after combination chemotherapy with adriamycin, cyclophosphamide and vincristine. Cancer Chemother Rep 58: 365–370
7. Graham-Pole J, Lazarus HM, Herzig RH, et al (1984) High-dose melphalan therapy for the treatment of children with refractory neuroblastoma and Ewing's sarcoma. Am J Pediatr. Hematol Oncol 6: 17–26

8. Hartmann O, Scopinaro M, Tournade MF, et al (1983) Neuroblastomes traités à l'Institut Gustave-Roussy de 1975 à 1979. Arch Fr Pediatr 40: 15–21
9. Hartmann O, Pein F, Beaujean F, et al (1984) High-dose polychemotherapy with autologous bone marrow transplantation in children with relapsed lymphomas. J Clin Oncol 2: 979–985
10. Hartmann O, Beaujean F, Bayet S, et al (1985) Autologous bone marrow transplantation: role of cryoperservation, number of cells infused and nature of high-dose chemotherapy. Eur J Cancer 21: 53–60
11. Helson L (1979) Investigational chemotherapy of neuroblastoma. J Fl Med Assoc 66: 284–287
12. Kretschmar CS, Frantz CN, Rosen EM, et al (1984) Improved prognosis for infants with stade IV neuroblastoma. J Clin Oncol 2: 799–803
13. Lazarus HM, Herzig RH, Graham-Pole T, et al (1983) Intensive melphalan chemotherapy and cryopreserved autologous bone marrow transplantation for the treatment of refractory cancer. J Clin Oncol 1: 359–367
14. Maraninchi D, Abecassis MP, Gastaut TA, et al (1983) High-dose melphalan and autologous bone marrow transplant for relapsed acute leukemia. Cancer Chemother Pharmacol 10: 109–111
15. Maraninchi D, Pico JL, Hartmann O, et al (1985) High-dose melphalan with or without marrow transplantation: a study of dose-effect in patients with refractory and/or relapsed acute leukemias. Cancer Treat Rep (to be published)
16. McElwain TJ, Hedley DW, Gordon MY, et al (1979a) High dose melphalan and non-cryopreserved autologous bone marrow treatment of malignant melanoma and neuroblastoma. Exp Haematol 7 [Suppl 5]: 360–371
17. McElwain TJ, Hedley DW, Burton G, et al (1979b) Marrow autotransplantation accelerates haematological recovery in patients with melanoma treated with high-dose melphalan. Br J Cancer 40: 72–80
18. Ninane J, Pritchard J, Malpas JS (1981) Treatment of advanced neuroblastoma: does adriamycin contribute? Arch Dis Child 50: 544–548
19. Pritchard J, McElwain TJ, Graham-Pole J (1982) High-dose melphalan with autologous bone marrow for advanced neuroblastoma. Br J Cancer 45: 86–94
20. Thomas ED (1982) The use and potential of bone marrow allograft and whole-body irradiation in the treatment of leukemia. Cancer 50: 1449–1454
21. Thomas ED, Storb R (1970) Technic of human marrow grafting. Blood 36: 507–515
22. Zucker JM (1974) Retrospective study of 462 neuroblastoma treated between 1950 and 1970. Maandschr Kindergeneesk 42: 369–385

Received June 6, 1985/Accepted August 21, 1985