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Original Paper

Stage IV Neuroblastoma in Patients Over 1 Year of Age at Diagnosis: Consolidation of Poor Responders with Combined Busulfan, Cyclophosphamide and Melphalan Followed by *In vitro* Mafosfamide-Purged Autologous Bone Marrow Transplantation

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In an attempt to improve the poor prognosis of poor responders with stage IV neuroblastoma, a new combined high-dose chemotherapy conditioning regimen was tested. Event-free and overall survival, as well as the incidence of complications, were analysed. Twenty-five children aged 12–146 months at diagnosis entered this study. All were in complete remission (CR) at the time of high-dose chemotherapy. Two or three different protocols had been necessary for them to achieve a CR. High-dose chemotherapy consisted of a combination of busulfan (600 mg/m^2), cyclophosphamide (4400 mg/m^2) and melphalan (140 mg/m^2). It was followed by autologous bone marrow transplantation (ABMT). The bone marrow graft was purged *in vitro* with mafosfamide. The probability of event-free survival (EFS) at 5 years post-ABMT was 34%, compared to <8% in a historical series. Toxicity was severe but manageable and 2 complication-related deaths were observed. Veno-occlusive disease was the most frequent extrahaematopoietic complication encountered, but its outcome was always favourable. By using a very intensive conditioning regimen consisting of a combination of three alkylating agents, the EFS of poor responders with metastatic neuroblastoma was improved and similar to that of good responders. When compared with a previously published similar series of patients, the improvement in survival appears probably related to intensification of the conditioning regimen. © **1997 Elsevier Science Ltd.**

Key words: stage IV neuroblastoma, autologous bone marrow transplantation, alkylating agents, busulfan–cyclophosphamide–melphalan
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INTRODUCTION

DURING THE last decade, high-dose chemotherapy followed by autologous bone marrow transplantation (ABMT) has been extensively used as consolidation therapy in stage IV neuroblastoma. The vast majority of BMT series concerned children over 1 year of age at diagnosis given that children under 1 year of age at diagnosis did not need BMT due to their better prognosis [1, 2]. In a previous study [3] we showed that the quality of response to primary chemotherapy was a prognostic factor. The poor responders, namely patients who needed more than one conventional chemotherapy protocol to enter complete remission, had a very poor outcome following high-dose chemotherapy and ABMT. We investigated a new combination of three alkylating agents as consolidation therapy with autologous bone marrow rescue in these poor responders. We report here the results obtained with this combination in 25 children with metastatic neuroblastoma over 1 year of age at diagnosis.

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PATIENTS AND METHODS

Between 1987 and 1993, 25 children were considered eligible for this study. All had stage IV neuroblastoma according to the ENSS classification [4] and were over 1 year of age at the time of diagnosis. Their median age at diagnosis was 41 months (range 12–146) and the male to female ratio was 17:8. The primary tumour was abdominal in 23, thoracic in 1 and of an unknown origin in 1. All patients had metastases at diagnosis. The disease involved the bone marrow in 24 cases and the bone in 21. Primary tumour diameter was >10 cm in 8 cases and <10 cm in 17. Disease was detectable in locoregional lymph nodes at imaging in 17/25 children.

For first-line chemotherapy, 2 patients were treated with four courses of a combination of vincristine, cyclophosphamide and doxorubicin (CADO) [3]. The 23 remaining patients were treated with four alternating courses of CADO and cisplatin–etoposide [5]. After these first four courses, all 25 patients exhibited detectable residual metastatic disease and required a second (22 patients) or third (3 patients) drug combination. Second-line chemotherapy was usually a combination of carboplatin and etoposide [6] and third-line chemotherapy was a combination of high-dose cyclophosphamide and etoposide [7]. After completion of second- or third-line chemotherapy, all patients were free of metastasis and fulfilled the definition of poor responders (complete response at metastatic sites slowly obtained).

Surgical excision was attempted in all patients with a detectable primary. Excision was macroscopically complete in 19 patients and macroscopically subtotal in 5. As a result of these treatments, 18 patients entered complete remission (CR) with no detectable disease and 7 had a complete disappearance of all metastatic signs but a small macroscopic residual primary tumour. Their response to primary therapy was termed 'very good partial remission' (VGPR).

Remission was defined according to the following criteria. CR was defined as (1) a completely normal clinical examination; (2) normalisation of urinary catecholamine metabolites; (3) a normal mIBG scan; (4) normal bone marrow as assessed by extensive staging [8] and macroscopically complete excision of the primary assessed by surgery and postoperative CT scan. VGPR was defined according to the same criteria for metastases and any residual primary tumour of <5% of the initial size.

The chemotherapy regimen consisted of a combination of busulfan, cyclophosphamide and melphalan. On days -8, -7, -6, -5, busulfan (150 mg/m^2/d) was administered orally (37.5 mg/m^2 every 6 h). On days -4 and -3, cyclophosphamide (2200 mg/m^2/d) was infused intravenously over 3 h. On day -2, melphalan (140 mg/m^2) was administered as an I.V. bolus through the central venous line. During the 7 days of chemotherapy, hydration (3 l/m^2/d) was performed with 5% dextrose and the usual concentration of electrolytes. Thawed bone marrow was infused on day 0. No patient received post-ABMT therapy before a relapse.

When the bone marrow was harvested, all patients were in CR or VGPR. Samples of harvested bone marrow were extensively screened for residual tumour cells using cytological techniques, as previously described [8]. All were free of detectable tumour cells. The techniques used for bone marrow harvesting and *in vitro* purging with mafosfamide have been described elsewhere [3].

Supportive care was performed as described elsewhere [3].

The criteria used to define the intensity of complications in critical organs have been described elsewhere [3].

After discharge, patients were seen at least every month during the first 6 months post-transplantation and every 3 months thereafter. Serial evaluation of their status was carried out by clinical examination, urinary catecholamine metabolite determinations, abdominal CT scan, mIBG scan and extensive bone marrow staging.

RESULTS

As of May 1996, 9 patients are alive in continuous CR. The median follow-up postdiagnosis is 82 months (range 57–127). Thirteen patients relapsed at a median of 12 months post-ABMT (range 4–23 months). Two complication-related deaths occurred. Overall, the probability of disease-free survival at 5 years post-ABMT was 34% (Figure 1). No prognostic factor was significant, particularly at age under 2 years.

The median duration of granulopenia ($<0.5 \times 10^9$/l) and thrombocytopenia ($<50 \times 10^9$/l) was 30 days (range 12–110) and 126 days (range 15–469), respectively. Patients were given a median of six RBC transfusions (range 2–26) and a median of 20 platelet transfusions (range 4–58).

Seven cases of haemorrhagic cystitis were observed at the beginning of the study. All recovered without late effects. However, as soon as effective prophylaxis was prescribed, this complication no longer occurred. Gastrointestinal toxicity consisted mainly of moderate to severe mucositis (18/25 patients, 72%). Diarrhoea, grade $\geq$2, was less frequent (9/25 patients, 36%). Two cases of severe gastrointestinal haemorrhage were observed which resolved rapidly without sequelae. Liver toxicity was frequent. Thirteen patients (52%) had transient liver abnormalities. Seven of these patients had typical signs of veno-occlusive disease [10]. All these hepatic complications resolved completely without sequelae. Four patients experienced generalised seizures occurring during high-dose chemotherapy, with clonazepam prophylactic therapy the complication was no longer observed.

All patients experienced fever for a median duration of 19 days (range 2–59). Two of the 25 patients had bacteraemia (8%) (gram-negative bacilli [1], *candida albicans* [1]). Seven cases of herpes zoster were observed in 6 patients. They occurred at a median time of 67 days post-BMT (range 40–135). Two complication-related deaths occurred (8%). One was related to interstitial pneumonitis of an unknown origin, the

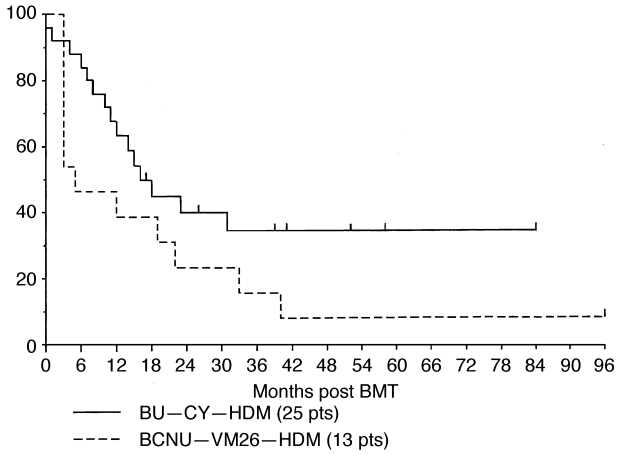

Figure 1. Comparison of event-free survival according to the conditioning regimen (poor responders with neuroblastoma): BU, busulfan; CY, cyclophosphamide; HDM, high-dose melphalan; BCNU, carmustine; VM26, teniposide.

other was the consequence of a cerebral haemorrhage occurring during thrombocytopenia and acute metabolic disorders.

DISCUSSION

In a previous study [3] we demonstrated that post-BMT event-free survival was lower in poor responders than in good responders to conventional chemotherapy. With this new combination of three alkylating agents as the conditioning regimen, the event-free survival of these poor responders is higher. As shown in Figure 1, under the previous conditioning regimen (carmustine, teniposide, melphalan), the 5-year post-BMT probability of EFS was <10% and is 34% with the present regimen. This difference is not statistically significant ($P = 0.06$), but the probability of relapse or death was 2.02 times higher in the previous group of patients (log-rank test). However, this is a historical comparison and some other factors could play a role in this difference. These two series of patients were not statistically different in terms of sex, age and status at the time of bone marrow transplantation. However, the principal characteristics of previous conventional chemotherapy were different between the two series. The patients conditioned with the carmustine, teniposide, melphalan combination had received a significantly longer conventional chemotherapy with a significantly lower number of drugs. The prognostic significance of these differences is difficult to evaluate. Compared to the second series, the lower number of drugs used in the first series could be an adverse prognostic factor, but the longer duration of conventional chemotherapy in the same series is considered a favourable prognostic factor. It has been previously demonstrated that the later the consolidation was performed, the higher the probability of survival [11].

The difference in EFS between these two series is not explained by the difference in the toxic death rate. The prognostic significance of response to primary chemotherapy has been recognised in other studies [12]. Analysis of the EBMT registry has also demonstrated that detectable bone metatases after the completion of first-line chemotherapy is a poor prognostic factor [13]. Despite the fact that this population was a selected group of poor responders to first-line chemotherapy, EFS in patients given this three alkylating agent combination appears comparable to that of series of good responders published. Thus, these results compare favourably with those of other conditioning regimens, especially those containing total body irradiation (TBI) [11–15].

The choice of these three alkylating agents for this combined high-dose chemotherapy regimen was based on previous studies of high-dose chemotherapy in neuroblastoma [11]. Several phase II studies of high-dose melphalan have already been published. The overall response rate observed in these studies was 55% [16]. Combination busulfan and cyclophosphamide has been widely used in acute myeloblastic leukaemias but few phase II studies have been performed with this combination in paediatric solid tumours [17]. We did, however, observe a 30% response rate in refractory neuroblastoma treated with this combination in a previous series [18]. Combination busulfan and melphalan has been tested in several malignancies such as malignant lymphomas [19, 20], leukaemia [21] and solid tumours in adults [20] and children [22]. The combination of these three alkylating agents has been already published in adult patients treated for haematological malignancies [23], in whom critical organ toxicity was particularly high.

The dose chosen in our study for each agent was based on that administered in previous studies. We have previously demonstrated that 600 mg/m^2 (cumulative dose) was the optimal dose of busulfan to be used in children to obtain an area under the curve similar to that observed in adults treated with 16 mg/kg [24]. The cyclophosphamide dose used in this combination is that described by Tutschka and associates [25]. Finally, the 140 mg/m^2 dose of melphalan is that commonly used for this drug for high doses, although higher doses attaining 200 mg/m^2 have been used [26]. It was used at this dose level in the unique randomised study of this drug in neuroblastoma patients [27].

This regimen was highly myelotoxic. However, engraftment was complete in all patients. The long duration of aplasia is related to *in vitro* purging techniques, as previously documented [28]. Moreover, the high incidence of hepatic veno-occlusive disease played a major role in the number of platelet transfusions [29]. The use of haematopoietic growth factors (G-CSF, GM-CSF) during the post-BMT period should now shorten this aplastic phase. The incidence of haemorrhagic cystitis was high at the beginning of the study. Both busulfan and cyclophosphamide are known to provoke urothelial damage [30], but with simple prophylactic measures this complication no longer appeared in the subsequently treated patients.

Liver toxicity, especially veno-occlusive disease, was the most specific complication of this regimen. In a previous study, we demonstrated that both high doses of busulfan and the use of three alkylating agents were major risk factors for the occurrence of VOD [31]. In this series, no patient died of liver toxicity and all recovered completely. However, this complication did lead to prolonged hospitalisation and a high consumption of platelet transfusions.

Generalised seizures are a well-known complication of high-dose busulfan. We have previously shown that it is dose related and can be prevented in routine practice by the concomitant administration of clonazepam [32].

Transplant-related mortality was acceptable (8%) and in the same range as that of our previous study. However, given the duration of aplasia and the incidence of organ complications, intensive supportive care and expert nursing were necessary to control the morbidity of this regimen. Patients remained hospitalised for a median duration of 57 days (range 33–134).

With a median follow-up of 56 months post-BMT, the disease-free survival of these poor responders with neuroblastoma is an improvement compared with the results of our previous experience. The high intensity of the conditioning regimen used appears to be a favourable prognostic factor, but more accrual is necessary to definitively demonstrate its role in this improvement. Intensive supportive care limited the complication-related death rate, but the morbidity, especially related to the incidence of VOD, was high. This incidence could possibly be decreased by monitoring busulfan plasma levels in children so that the tolerance of this regimen can be improved in the future [33].

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