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D–CECaT: a breakthrough for patients with neuroblastoma

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In view of the high relapse rate following chemotherapy for patients with advanced neuroblastoma (NB) and primitive neuroectodermal tumors (PNET), we designed a novel chemotherapy program which incorporated the iron chelator deferoxamine. The purpose of the deferoxamine was to sensitize the cells to standard chemotherapy. The D–CECaT regimen contained (in mg/m²): deferoxamine 4500 during days 1–5; cyclophosphamide 600 mg over days 6 and 7; etoposide 300 mg over days 7 and 8; carboplatin 100 mg over days 7 and 8; and thiotepa 30 mg over days 6–8. Between October 1989 and May 1992 we entered 23 advanced NB and two PNET patients. Sepsis occurred in four courses, nausea and vomiting in 30 courses, and 50 courses required blood and platelets. Responses observed in previously untreated patients with stage III NB: six out of six CR (17 + to 41 + months), with stage IV NB, nine out of 11 CR (14 + to 28 + months), two out of 11 VGPR (22 + months), with stage IV PNET two out of two CR (1 + to 35 + months). With previously treated and failed stage IV NG, two out of six VGPR for 19 + and 20 months, and four out of six PR 1, 8, 9 and 11 months. Median survival for 19 new patients was 22 + months (6 to 41 + months; two patients in CR died at 7 months during adjuvant autologous marrow transplant). In conclusion, D–CECaT is an effective initial cytoreductive regimen for advanced stage NB/PNET patients. Additional patients and studies are required to determine its use as an alternative to autologous bone marrow transplantation.

Key words: D–CECaT, neuroblastoma, primitive neuroectodermal tumor.

Introduction

Neuroblastoma (NB), the most common extracranial solid tumor of childhood, occurs as advanced disease at diagnosis in nearly 50% of infants and 70% of children.[1] In infants it is characterized by early stage disease which may be one reason for the better outcome of patients with a diagnosis during the first year of life. In children, the advanced disease state is characterized by greater metastatic potential and limited sensitivity to cytoreductive drugs. Despite improvement in the duration and incidence of progression-free survival with radiation therapy and multiagent ablative chemotherapy, in low or very high doses followed by autologous bone marrow transplantation (ABMT), long-term survival of children remains less than 20%.[2,6–11] Philip and colleagues have in fact reported an overall 6 year actuarial survival of only 10% in a trial of aggressive chemotherapy followed by ABMT in children with advanced NB.[3,4]

Clearly an alternative or complement to standard drug selection, schedules and dosages used in experimental trials was required. Cognizant of the central role of iron in cell survival and replication, we designed and evaluated a new treatment regimen consisting of deferoxamine (DFO), an iron chelating agent, followed by a combination of four cytotoxic drugs. Incorporating an iron chelating agent was based on the anti-proliferative effects of DFO in NB cells and patients with disseminated NB. Alone, DFO caused a decrease in tumor infiltration of marrow after 5 days of treatment.[16] DFO-induced cytostatic effects have been associated with an arrest of cell progression in the post-mitotic G_1/DNA synthesis phase of the divisional cycle.[5,24] However, from *in vitro* data, a combination of cytostasis and lysis could occur. We chose the four drugs based on previous studies with carboplatin (CBPCA)–etoposide (VP-16)[17] and carmustine–CBDCA–VP-16–thiotepa (TT) combinations.[18] We substituted cyclophosphamide for carmustine because of a better therapeutic profile and much less irreversible myelotoxic activity of the former. Further, we wished to avoid carmustine-induced pulmonary toxicity. Thiotepa was included because of its steep dose–response curve and synergistic activity with cyclophosphamide.[19] *In vitro* studies of

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the combination of cyclophosphamide and thiotepa revealed supra-additive killing of MCF-7 human breast cancer cells.[19] In MCF-7 tumor-bearing mice, thiotepa at 5 mg/kg, cyclophosphamide at 100 mg/kg or both drugs combined were studied. Thiotepa caused a delay in tumor growth of 2.7 days, cyclophosphamide for 12.5 days and the combination for 28.8 days.[19,20]

The strategy of scheduling DFO before alkylating agents was based on several assumptions. The first is that the major mechanism of action of DFO, i.e. iron chelation, leads to some degree of intracellular depletion of iron-dependent enzymes such as ribonucleotide reductase and catalase. Such changes could persist for enough time to limit repair of damage caused by the CECaT regimen. The second is that increased serum iron and saturation of transferrin immediately follow chemotherapy,[25] limiting iron transport across cell membranes. Adding desferal at this late point in time will probably contribute little towards intracellular iron depletion.

This study was performed to determine the safety as well as cytoreductive efficacy of this five agent regimen, termed D–CECaT. The regimen includes DFO, cyclophosphamide (CPM), VP-16, CBDCA and TT. We targeted peripheral neural tumored patients [NB and primitive neuroectodermal tumor (PNET)] who were either newly diagnosed or who had tumors recurrent or refractory.

Materials and methods

From October 1989 to May 1992, 25 patients with a median age of 2.4 years (range 0.8–12.9) were entered into this study. The patients with NB were staged according to the International Neuroblastoma Staging System (INSS).[21] The main characteristics of these patients are listed in Table 1. They included 25 patients; two with PNET, six with stage 3, 11 with stage 4 and six pre-treated patients with stage 4 NB. Eligible patients were required to have: histologically confirmed stage 3 or 4 NB or PNET; measurable disease; life expectancy of at least 4 weeks; normal liver function, defined as serum bilirubin <1.0 mg/dl and aspartate aminotransferase (AST) <30 U/l; normal renal function, defined as blood urea nitrogen (BUN) <20 mg/dl and serum creatinine <1.0 mg/dl; and, if previously treated, recovery from toxicity. Informed consent was obtained from parents according to institutional guidelines.

The treatment schedule consisted of DFO (150 mg/kg/day) administered intravenously (i.v.) (maximum daily dose 600 mg) in normal saline (250–500 ml/m^2) for 8 h on days 1–5 followed by CPM (300 mg/m^2/day) on days 6 and 7, VP-16 (150 mg/m^2/day), CBDCA (500 mg/m^2/day) on days 7 and 8, and TT (10 mg/m^2/day) on days 6–8. Courses were given every 28 days, providing an adequate hematologic recovery ($>1\,000$ granulocytes/μl and 100 000 platelets/μl) had taken place. Prophylactic antiemetic therapy was given in all courses. Hematologic, gastrointestinal, hepatic, renal and neurological toxicities were monitored according to standardized Eastern Cooperative Oncology Group (ECOG) criteria.[22] Serial audiometric testing was carried out in all patients prior to therapy and after four courses. In all patients a double lumen central venous catheter was inserted prior to chemotherapy. Patients were evaluated after two and four courses by computed tomography (CT) or magnetic resonance imaging (MRI), two bone biopsies and four marrow aspirates. Patients were also evaluated with ^{131}I-Meta Iodo Benzyl Guanidine (^{131}I-MIBG) prior to therapy and after surgery. The criteria for response were as follows: complete response (CR) the disappearance of all measurable disease for a minimum of 4 weeks; very good partial response (VGPR), more than 90% reduction of the primary tumor, with disappearance of all metastatic lesions (except bone) and improvement of all pre-existing lesions for at least 4 weeks; partial response (PR), more than 50% reduction in the product of the two greatest perpendicular diameters of all measurable lesions for a minimum of 4 weeks; minor response (MR), less than 50% but more than 25% reduction of the two greatest perpendicular diameters of all measurable lesions or more than 50% reduction for less than 4 weeks; stable disease (SD), no significant change in any disease parameter for a minimum of 4 weeks; progressive disease (PD), increase in any measurable tumor or the appearance of any new lesion. In all patients tumor size was measured after two and four courses by serial CT and/or MRI scans. Bone marrow involvement of the iliac crests was evaluated by two needle biopsies and four aspirates. The presence of tumor cells was determined by using a panel of monoclonal antibodies against neuroectodermal antigens.[23]

Results

All enrolled patients were evaluable for toxicity and all but two were evaluable for response to therapy.

Table 1. Patient characteristics and response to therapy

Patient	Age (years)	Stage	Courses 2	Courses 4	Surgery	Survival (months)
1	2.10	NB 3	CR	CR		39 +
2	3.5	NB 3	PR	CR		39 +
3	3.0	NB 3	PR	CR		41 +
4	3.3	NB 3	PR	PR	CR	19 +
5	1.6	NB 3	MR	PR	CR	17 +
6	1.4	NB 3	PR	PR	CR	20 +
7	2.4	NB 4	PR	VGPR	CR	7[a]
8	1.2	NB 4	PR	PR	CR	15
9	1.6	NB 4	CR	CR		28 +
10	4.0	NB 4	PR	PR	VGPR	22 +
11	1.9	NB 4	PR	PR	CR	21 +
12	3.1	NB 4	PR	PR	VGPR	22 +
13	1.1	NB 4	CR	CR		25 +
14	1.6	NB 4	PR	PR	CR	6[b]
15	2.10	NB 4	PR	VGPR	CR	23[c]
16	1.5	NB 4	PR	VGPR	CR	7[a]
17	2.10	relapse	PR	PR		11
18	6.4	relapse	1 course			DOD
19	0.8	relapse	VGPR			20
20	2.4	relapse	PR			9 +
21	6.2	relapse	PR			8
22	8.0	relapse	PR	PR	VGPR	19 +
23	0.8	NB 4		CR		14 +
24	2.7	PNET	PR	PR	CR	31 +
25	12.9	PNET	PR	PR	CR	35 + [d]

[a]Died in CR during ABMT.
[b]Died in CR after surgery.
[c]Underwent ABMT.
[d]In second CR at 22 months.

They received a total of 86 courses, with a median of four courses per patient (Table 1). No drug-related deaths occurred.

All but two patients achieved an objective response following the first two courses. One previously treated chemotherapy resistant patient died from progressive disease after the first course (Table 1). In patients without previous treatment the overall response rate (CR + PR) was three out of six CR after four courses and six out of six CR after surgery for patients with NB stage 3; and two out of 10 CR, two out of 10 VGPR and six out of 10 PR after four courses; seven CR, two VGPR and one PR after surgery for patients with NB stage 4. Two of these patients in CR died of causes unrelated to their tumor after an autologous bone marrow transplant in another center; one patient died in CR at 1 month for complications secondary to surgery. For two patients with PNET: one PR and one CR after four courses, and two out of two CR after surgery. One of these patients relapsed after 21 months and is now in second CR. In six pre-treated patients the overall response rate was one VGPR, four PR, one NE (only one course) after two courses, and two PR after four courses; only one patient is alive in PR after surgery at 9 months.

Toxicity

Transitory myelosuppression was found to be the major toxic effect, as shown in Table 2. Grade 4 leukopenia (<1 000 WBC/μl) occurred in 67% of the courses; the median time to reach nadir leukocyte counts (range 0–2 000) after initiating

Table 2. Toxicity observed: occurrences as percentage of 86 administered D–CECaT courses

Toxicity	ECOG grade				
	0	1	2	3	4
Hematologic					
Leukopenia	0	0	3	30	67
Thrombocytopenia	0	1	12	27	60
Anemia	0	3	17	80	—

D–CECaT treatment was 19 days (range 9–18). Grade 4 thrombocytopenia (platelets $<25\,000/\mu l$) occurred in 60% of the courses; the median time to nadir thrombocytopenia (range 6 000–200 000) in all courses was 14 days (range 7–33) after initiating treatment. Grade 3 anemia [requiring packed red blood cell (RBC) transfusion] occurred in 80% of the courses; the median time to the nadir in hemoglobin concentration (6.4–9.6) in all courses was 12 days (range 4–23) after initiating D–CECaT treatment. The median interval between courses was 41 days for the pre-treated patients (range 30–60) and a median of 30 days for the untreated patients (range 24–57). Based on the degree of myelosuppression, virtually all patients required supportive care. Culture-negative fever for which intravenous broad spectrum antibiotic therapy was given was observed in 60 out of 86 courses. Six documented sepsis caused by *Klebsiella pneumoniae*, one *Pseudomonas aeruginosa*, one *Staphylococcus epidermidis* and three *Enterobacter cloacae* were observed. All responded to antibiotic therapy. A total of 114 packed RBC transfusions were given in 86 courses with a median of 1.3 RBC/course; while 247 platelet transfusions were administered with a median of 2.9 platelet transfusions/course. Allergic reactions were not observed. Myelosuppression appeared to be more severe in pre-treated patients. Non-hematologic toxicities were rarely observed. Nausea and vomiting were easily controlled by ondansetron. Slight ephemeral elevations of hepatic enzymes were observed. Renal toxicity and electrolyte disturbances were not encountered. No acute central or peripheral neurologic abnormalities were noted. Serial audiometric testing performed in 11 untreated patients prior to starting chemotherapy and after four courses of chemotherapy revealed four patients with 10–20 decibel losses in the high frequencies.

Discussion

The major adverse effects of this regimen were limited to myelosuppression. Minor adverse events such as liver enzyme changes were ephemeral. A larger group of patients is required to determine the incidence of ototoxicity. Pulonary toxicity had not been observed in patients treated with these dosages of DFO in 86 courses. Reports of a DFO 'pulmonary' syndrome has engendered focus on the potential[24] and speculation on the etiology of the pulmonary syndrome.[25] Because of the very good quality and duration of responses seen in these patients, we intend to test this iron chelation/chemotherapy strategy in a larger group of patients with advanced NB and other cancers. Assuming these data are reproducible, it still has to be proven if improving the extent of cytoreduction in previously untreated NB patients caused by the addition of DFO to a cytotoxic regimen will be followed by very significant increases in long-term disease-free survival.

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