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Deferoxamine Followed by Cyclophosphamide, Etoposide, Carboplatin, Thiotepa, Induction Regimen in Advanced Neuroblastoma: Preliminary Results

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Based upon phase I and II studies of deferoxamine alone and in combination with cytotoxic agents cyclophosphamide, etoposide, carboplatin, and thiotepa (D-CECaT), we initiated a single arm multicentre trial in 1992 for advanced neuroblastoma. 57 of 65 patients who entered the trial were evaluable. Following 4 courses of the D-CECaT, almost all the patients underwent surgery. Toxicity was moderate and mainly reversible myelosuppression. The post-surgically defined responses in stage 3 high risk, stage 4 moderate risk and stage 4 high risk patients included 24 complete responses, 26 partial responses, and 3 minor responses, and 4 patients had progressive disease. These patients are being followed to determine the impact of this programme on their overall survival.

Key words: deferoxamine, neuroblastoma, cytoxan, etoposide, carboplatin, thiotepa, D-CECaT, iron chelation
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INTRODUCTION

STAGE IV neuroblastoma in children over 1 year of age at diagnosis is the most common childhood malignancy under the age of 6 years[1]. The limitations of surgery and radiotherapy have been clearly defined in treatment strategies of metastatic neuroblastoma, where emphasis and dependence on chemotherapy have been foremost.

Patients treated prior to 1980 had an expected 10% survival rate 3 years after diagnosis using conventional chemotherapy regimens including cyclophosphamide (CPM), doxorubicin (DXR), and vincristine (VCR). Survival statistics have not changed following the addition of cisplatin (CDDP) and epipodophyllotoxins (etoposide, VM-26). The latter two have had a significant impact on initial response rates, and improved the duration of progression-free survival when incorporated into multi-agent chemotherapeutic regimens [2, 3]. High-dose chemotherapy followed by bone marrow or peripheral blood stem cell rescue [4–8] or meta-iodobenzylguanidine (MIBG) as consolidation treatment appear to have short-term antitumoral effects in selected responding patients. The intrinsic limited sensitivity of advanced neuroblastoma to these cytoreductive regimens is manifested by the failure of any of these regimens to produce true complete remissions. This emphasises a need for improved therapeutic strategies. Improvements have been few because selecting the most appropriate drugs [9, 10], schedule of administration and dosages have been based on a combination of intuitive and empirical reasoning combined with fragmentary pharmacological data. Within this framework, and using limited data from *in vitro* studies suggesting an unusual sensitivity to iron depletion by cultured neuroblastoma cells, we designed a pilot clinical study to determine the safety and the antitumoral effects of a single course of deferoxamine (DFO), an iron chelating agent. Of 9 patients, we observed reduction of bone marrow involvement in 7, decrease of tumour mass in one and no effect in another [11]. These initial observations were incorporated into the design of a second regimen consisting of the combination of four cytotoxic agents (cyclophosphamide, etoposide, carboplatin and thiotepa) plus deferoxamine as the iron chelating agent (D-CECaT) [12]. The presumed mechanism of action of DFO was that its iron chelating activity inhibited neuroblastoma cell ribonucleotide reductase and other intracellular enzymes having iron as a cofactor, leading to reduced DNA synthesis repair, and enhanced requirements for intracellular iron. DFO-treated neuroblastoma cells would have increased transferrin receptors on the cell surface. This pharmacological cell sensitisation based on iron needs, and altered cell cycling events manifested as a sort of "synchronisation", suggested that adding DNA-based cytotoxic agents would be most effective after DFO.

The rationale for the cytotoxic drugs chosen was based on previous phase II studies with carboplatin–etoposide [13, 14]

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Table 1. ICGNB Staging criteria

Stage	Criteria
1	Macroscopically radical excision of primary tumour. Regional lymph nodes negative for tumour infiltration.
2	(a) Primary tumour excised with minimal local residue (presumably less than 2 ml). (b) Primary tumour excised radically or with minimal residue; regional lymph nodes positive for tumour infiltration. (c) Primary tumour excised radically or with minimal residue; tumoral infiltration of intervertebral foramina with or without clinical signs of spinal cord compression. (d) Primary tumour excised radically or with minimal residue; accidental tumour rupture before or during surgery.
3	Primary tumour stated inoperable on the basis of clinical and imaging evaluation; or primary tumour excised with greater than minimal residue.
4	Remote disease involving the skeleton, organs, soft tissues, and distant lymph node groups.
5	Patients aged 0–11 months who would otherwise be stage 1 or 2, but who have remote disease confined to liver, skin, or bone marrow (in any combination) and have no evidence of bone metastases.

and carmustine–carboplatin–etoposide–thiotepa combinations [15–18]. The combination of CPM and thiotepa has been shown to produce supra-additive killing *in vitro* of MCF–7 human breast cancer cells [17].

All 13 patients treated in the pilot D-CECaT study and the nine initial patients responded without side-effects attributable to DFO. Based upon these two studies, the Italian Cooperative Group for Neuroblastoma (ICGNB) decided to evaluate the toxicity and the antitumour effects of the D-CECaT regimen as front line therapy in a multicentric study.

PATIENTS AND METHODS

From March 1992 to October 1993, 65 consecutive patients with a median age of 38 months (range 6–162) were entered into the study. 57 patients were evaluable for response. Patients were staged according to the ICGNB, (Table 1) an affiliate of the Italian Association for Paediatric Haematology-Oncology (AIEOP). The main characteristics of these patients are listed in Tables 2–4. Eligibility criteria included histologically confirmed Stage 3 or 4 neuroblastoma or unequivocal presence of tumour cells in the bone marrow in association with abnormal urinary excretion of catecholamine metabolites. The existence of bone marrow infiltration was determined by a minimum of 4 site iliac bone biopsies, and tumour localisation in other bones with Tc 99 m methylene diphosphonate and/or MIBG scans. The presence of tumour cells was investigated by histological examination and an indirect immunofluorescence assay using a panel of monoclonal antibodies against neuroectodermal antigens[19]. Exclusion criteria included age under 6 months, prior chemotherapy, and concomitant major organ or metabolic dysfunctions, defined as serum bilirubin < 1.0 mg/dl and aspartate aminotransferase (AST) < 30 U/I; normal renal function, defined as blood urea nitrogen (BUN) < 20 mg/dl and serum creatinine < 1.0 mg/dl. Institutional informed consent was obtained from a parent or guardian before enrolment.

The treatment schedule consisted of intravenous (i.v.) DFO (80 mg/kg/day for 8 h on days 1–5, for the first course and then 150 mg/kg/day for 8 h for 5 days in subsequent courses). The dose in the first course was reduced because of complications thought to be related to iron deprivation secondary to tumour burden in 4 of the first 9 patients given 150 mg/kg/day as their first course. Administration of CPM (300 mg/m^2/day) followed on days 6 and 7, etoposide (100 mg/m^2/day) and thiotepa (10 mg/m^2/day) on days 6, 7, and 8, and carboplatin (500 mg/m^2/day) on days 7 and 8. The standardised Eastern Cooperative Oncology Group (ECOG) criteria were used to assess toxicity[20].

Responses were recorded according to the International Neuroblastoma Response Criteria (INRC). Complete response

Table 2. Neuroblastoma 92 — stage 3 (high risk)

Patient	Age (months)	Site	Response		
			After 2 courses	After 4 courses	After surgery
1	31	Abdominal	PR	PR	CR
2	12	Abdominal	MR	PR	CR
3	54	Thorax	PR	PR	VGPR
4	20	Abdominal	NR	NR	PR
5	51	Abdominal	NR	NR	NR
6	144	Abdominal	MR	PD	CR
7	21	Abdominal	NR	NR	PR
8	42	Abdominal	PR	PR	PR
9	91	Abdominal	CR	CR	CR

PR, partial response; CR, complete response; MR, minor response; NR, no response; VGPR, very good partial remission; PD, progressive disease.

Table 3. Neuroblastoma—stage 4 (intermediate risk)

Patient	Age (months)	Site	Response		
			After 2 courses	After 4 courses	After surgery
10	7	Thorax	PR	VGPR	CR
11	11	Abdominal	MR	MR	PR
12	8	Abdominal	PR	CR	CR
13	11	Abdominal	MR	PD	—
14	6	Abdominal	PR	PR	CR

Abbreviations as in Table 2.

Table 4. Neuroblastoma—stage 4 (high risk)

Patient	Age (months)	Site	Response		
			After 2 courses	After 4 courses	After surgery
15	32	Abdominal	PR	VGPR	CR
16	15	Abdominal	PR	CR	CR
17	74	Abdominal	MR	MR	MR
18	44	Abdominal	PR	VGPR	CR
19	22	Abdominal	PR	PR	CR
20	57	Abdominal	MR	PR	PR
21	24	Abdominal	NR	MR	CR
22	42	Abdominal	MR	PR	CR
23	33	Thorax	PR	PR	CR
24	58	Abdominal	PR	PR	PR
25	35	Abdominal	PR	PR	PR
26	15	Abdominal	PR	PR	VGPR
27	95	Abdominal	PR	PR	CR
28	29	Abdominal	PR	PR	CR
29	54	Abdominal	CR	CR	CR
30	56	Abdominal	PR	PD	PD
31	81	Abdominal	MR	CR	CR
32	26	Abdominal	PR	PR	PR
33	47	Abdominal	PR	PR	PR
34	32	Abdominal	PR	PR	PR
35	25	Thoracoabdominal	CR	CR	CR
36	67	Abdominal	NR	NR	PR
37	41	Abdominal	PR	PR	PR
38	50	Abdominal	PR	PR	PR
39	52	Abdominal	MR	PR	PR
40	32	Abdominal	NE	PR	PR
41	100	Abdominal	NE	MR	CR
42	162	Abdominal	NE	NR	MR
43	47	Abdominal	MR	MR	VGPR
44	37	Pelvic	PR	PR	CR
45	38	Abdominal	MR	PR	CR
46	16	Abdominal	CR	CR	CR
47	87	Abdominal	MR	MR	PR
48	69	Thorax	MR	PR	VGPR
49	49	Abdominal	PR	PR	PR
50	58	Abdominal	MR	MR	MR
51	112	Abdominal	PR	PR	PR
52	31	Abdominal	PR	PR	PR
53	23	Abdominal	MR	PR	PR
54	46	Abdominal	NE	PR	CR
55	52	Thoracoabdominal	MR	PR	PR
56	20	Abdominal	NR	MR	PD
57	22	Abdominal	MR	PR	VGPR

(CR) was defined as the disappearance of all evidence of disease for at least 4 weeks; very good partial remission (VGPR) was defined as > 90% reduction of the primary tumour, with disappearance of all metastatic lesions (except bone) and improvement of all pre-existing bone lesions for at least 4 weeks; partial response (PR) was defined as > 50% but < 90% reduction in all measurable lesions and improvement of all pre-existing bone lesions for at least 4 weeks; minor response (MR) was defined as < 50% reduction in any measurable lesion with < 50% reduction in any other lesions; no response (NR) was defined as < 50% reduction in any measurable lesion with < 25% increase in any other lesions; progressive disease (PD) was defined as an increase in any measurable lesion and/or the appearance of any new lesion and/or a previously negative bone marrow becoming positive for tumour involvement [21, 22].

RESULTS

There were 24 CRs, 5 VGPRs, 21 PRs, 3 MRs and 4 PD. For the Stage 3 high risk (HR) group, there were 4 CRs, 1 VGPR, 3 PRs and 1 NR; for the Stage 4 intermediate risk (IR) group, there were 3 CRs, 1 PR and 1 PD; and for Stage 4 HR, there were 17 CRs, 4 VGPR, 17 PRs, 3 MRs and 2 PDs. 5 of the first 9 patients (Nos 13, 17, 18, 19, 20) in this multicentre study developed non-fatal complications attributable to DFO. Four of these complications occurred within the first day of the first cycle given at 150 mg/kg/day. One developed severe bilateral interstitial pneumonia, sudden blindness and macroscopic haematuria. 2 developed bilateral interstitial pneumonia with dyspnoea and cyanosis, associated with pneumomediastinum, 2 other patients had less severe pneumonia; 1 of the 2 also had a uraemic-haemolytic syndrome. These patients did not receive further DFO, in accordance with the protocol, and for these reasons, the protocol was altered starting with the tenth patient. The dosage of DFO was reduced from 150 to 80 mg/kg/day, during the first cycle only. Since then, only one patient developed a moderately severe episode of bilateral pneumonia that resolved rapidly. All patients experienced a profound myelosuppression. Grade 4 leucopenia was observed in 70% of the courses; the median time to reach the nadir leucocyte count (range 0–600) in all courses was 18 days (range 14–22) after initiating treatment. Grade 4 thrombocytopenia (< 25,000 platelets/μl) was observed in 60% of the courses; the median time to reach the nadir platelet count (range 6000–86,000) in all courses was 18 days (range 12–26) after initiating treatment. Grade 3 anaemia (requiring packed RBC transfusion) occurred in 82% of the courses; the median time to reach the nadir haemoglobin concentration (range 6.2–9.7) was 18 days (range 8–24). Side-effects other than myelosuppression were rare. All patients experienced mild

gastrointestinal toxicity, such as nausea and easily controllable vomiting. Hepatotoxicity, nephrotoxicity and electrolyte disturbances were not observed in any patient. Allergic reactions were not recorded.

Of 9 patients with stage 3 HR neuroblastoma, one was operated on after the first course and achieved a CR. All the remaining 8 patients were operated on after the fourth course, and 3 achieved a CR, one a VGPR, three had PR, and one did not respond (Table 2). Of the 5 patients with stage 4 intermediate risk neuroblastoma, one had tumour progression and died 5 months after diagnosis. The other four had surgery, and three achieved a CR and one a partial remission after surgery (Table 3).

Of 43 patients with stage 4 high risk neuroblastoma, one patient had PD after four courses and was not operated upon. Of the 42 patients operated upon, one developed PD immediately following surgery, and there were 3 MRs, 17 CRs, 4 VGPRs, and 17 PRs (Table 4).

DISCUSSION

Compared with the initial pilot studies where toxicity related to DFO was not encountered [13], several patients in this multicentric study developed episodes of DFO-associated toxic events which caused the dose of DFO to be reduced. The pulmonary syndrome was important in all 5 patients. Acute blindness was followed by minimum recovery to date in one patient. The precise physiopathology of these toxicities are still not understood, although it appears to be associated with production of cytotoxic free radicals in lung parenchyma. Concerning the strategy of the D-CECaT protocol, it is based on *in vitro* experiments with human neuroblastoma cells where tumour cell death was observed after a minimum of 72 h constant exposure, and resistance to DFO after repeated exposures did not occur, and was not considered to present a factor in the clinical setting [23]. Further, the effectiveness of the strategy of scheduling DFO prior to alkylating agents was based on *in vitro* studies where post-treatment or concomitant treatment was less effective than pre-treatment DFO in neuroblastoma cells.

In summary, with 88% CR and PR responses determined after second look surgery, this regimen seems to be an effective inductive regimen for patients with advanced neuroblastoma. All patients exhibited clinically significant responses and serious but acceptable myelotoxic side effects. The next problem to address will be to determine whether these results are caused by the addition of DFO to the cytotoxic regimen. Presuming the iron chelation plus CECaT combination is critical to the response rate, it will be important to determine the actual long-term disease-free survival, and whether additional adjunctive therapy is required.

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