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Cyclophosphamide and VP 16-213 with Autologous Bone Marrow Transplantation. A Dose Escalation Study

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Abstract—*In 13 patients with therapy-resistant solid tumors the feasibility of high-dose cyclophosphamide (7 g/m²) in combination with increasing doses of VP 16-213 with autologous bone marrow transplantation was studied. Dose-limiting extramedullary toxicity appeared to be mucositis and occurred after 2.5 g/m². Two toxic deaths were observed in patients older than 55 yr. Responses were seen in eight out of nine evaluable patients. Two patients with ovarian cancer still have no signs of disease progression after 12+ months. High-dose cyclophosphamide (7 g/m²) can be combined with VP 16-213 1.5 g/m² without important extramedullary toxicity. Age is probably a limiting factor for this kind of therapy.*

INTRODUCTION

IN ANIMAL studies a steep dose–response relationship has been found for cytostatic drugs against animal tumor cells [1, 2]. Studies in man suggest that this model is also valid for human tumors. The response rate in small cell lung cancer (SCLC), carcinoma of the ovary (OC) and germ cell tumor (GCT) increases with intensification of chemotherapy [3–6].

A considerable dose escalation of cytostatic drugs results in progressive damage of normal tissue. One of the most frequently encountered side-effects is myelosuppression. The use of autologous bone marrow transplantation (ABMT) can prevent persistent bone marrow aplasia [7]. Cyclophosphamide and VP 16-213 (etoposide) are active agents in the above-mentioned tumors. As single agents both can be administered in high doses without severe extramedullary toxicity. Dose-limiting extramedullary toxicity of cyclophosphamide is cardiac failure caused by a hemorrhagic myocarditis. This is unlikely to occur below a dose of 240 mg/kg, but has been reported after single-agent cyclophosphamide at a dose of 180 mg/kg [8]. To date only one dose escalation study of VP 16-213 with ABMT has been reported. In this study the tolerated dose was 2.4 g/m², as in the majority of the patients at this dose level severe mucositis was observed [9].

In order to establish the feasibility of the combination of high-dose cyclophosphamide and high-dose VP 16-213 we started a phase I study with escalating doses of VP 16-213.

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MATERIALS AND METHODS

Patients

Pertinent data on 13 patients entering the study are given in Table 1. Patients were eligible up to the age of 70 yr. Entry criteria were bilirubin levels $\leq$25 mmol/l, leucocytes $\geq 3.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, serum creatinine levels $\leq$150 μmol/l (normal $\leq$106 μmol/l) and no signs of bone marrow invasion with tumor in bone marrow biopsy and smear.

Patients had to have a Karnofsky performance score $\geq$60. All patients had persisting or progressive disease after conventional treatment programs. Informed consent was obtained from all patients and the study was approved by the local medical ethical committee.

Table 1. Patient characteristics

Patient	Age (yr)	Sex	Tumor type	Previous therapy	Karnofsky score	Dose Cy (g/m^2)	Dose VP (g/m^2)	Response	Response duration (months)
1	29	F	OC	Cy, HMM, ADR, CDDP	80	7	0.9	CR	16+
2	42	F	OC	Cy, HMM- ADR, CDDP	80	7	0.9	NE	*
3	65	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB, PCI	60	7	0.9	NE	†
4	24	F	GCT	CDDP, VBL, BL	60	7	1.5	PR	1
5	51	F	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	70	7	1.5	PR	9+
6	38	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	80	7	1.5	SD	1
7	55	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	90	7	1.5	ED	ED day 13
8	40	M	GCT	CDDP, VBL, BL	80	7	2.0	PR	1½
9	24	M	GCT	CDDP, VBL, BL	90	7	2.0	PR	2
10	41	M	GCT	CDDP, VBL, BL	90	7	2.0	PR	1
11	22	M	GCT	CDDP, VBL, BL	80	7	2.5	PR	2
12	36	M	GCT	CDDP, VBL, BL	60	7	2.5	PR	2
13	57	M	SCLC	CDDP, VP, Cy, ADR, VCR, PCB	80	7	2.5	ED	ED day 22

ADR = adriamycin; BL = bleomycin; CDDP = *cis*-platinum; Cy = cyclophosphamide; VBL = vinblastine; VCR = vincristine; VP = VP 16-213; HMM = hexamethylmelamine; PCB = procarbazine; PCI = prophylactic cranial irradiation 30 Gy; SCLC = small cell lung cancer; GCT = germ cell tumor; OC = ovarian cancer; ED = early death; NE = non evaluable; CR = complete response; PR = partial response; SD = stable disease.

*No signs of progression after 12+ months.

†No signs of progression after 5+ months.

Bone marrow aspiration

Bone marrow was aspirated from the posterior iliac crests by multiple aspirations. The patients received mild sedation and general analgesia by diazepam 20 mg i.m. and meperidine 100 mg i.m. Lidocaine 1% was given as local anesthetic. A minimum of 2×10^8 nucleated cells/kg body wt was harvested in Hanks solution with HEPES buffer and Heparin, final concentration (FC) 150,000 IU/l. The marrow was centrifuged in an apheresis machine (Haemonetics Model 30S). The buffy coat was separated and resuspended in Hanks balanced solution (FC 200×10^6 nucleated cells/ml). This cell solution was dissolved (1:1) in 20% autologous plasma (FC 10%) and 20% DMSO (FC 10%) and placed in 5-ml freezing ampoules (Nunc). For freezing a Cryoson BV-4 liquid nitrogen controlled freezer was used at a rate of 1°C/min until -40°C. The ampoules were stored in liquid nitrogen.

Reinfusion was done after rapid thawing without washing. A blood filter was used to prevent infusion of possible clots.

Before marrow infusion the patients received prednisolone 60 mg i.v. and clemastine 2 mg i.v.

Cytostatic treatment

All patients received cyclophosphamide and VP 16-213. Cyclophosphamide was given as a 30-min infusion on three consecutive days. Mesna was given in a total dose of $4\ g/m^2$ in order to prevent hemorrhagic cystitis. VP 16-213 was dissolved in normal saline with a maximum concentration of 0.8 mg/ml. Two 1-hr infusions were given with a 12-hr interval on the same days as cyclophosphamide. The dose of VP 16-213 was increased if no dose-limiting extramedullary toxicity was seen in more than one out of three patients at that dose level (Table 1). The dose of cyclophosphamide given was $7\ g/m^2$, the highest dose that can be given without a high probability of cardiac toxicity [8].

The dose of VP 16-213 was escalated using a modified Fibonacci scheme [10], starting at $0.45\ g/m^2$ (unpublished observation).

The bone marrow was reinfused on day 7. At the moment of reinfusion of the bone marrow, VP 16-213 levels were measured. More extensive pharmacokinetic studies were done in two patients. VP 16-213 was determined by high-performance liquid chromatography with electrochemical detection [11].

Supportive care

Patients were treated in a single-person bedroom. Intravenous therapy was given either through a central venous Hickman catheter or through a needle inserted in an arteriovenous fistula [12].

All patients received prophylactic antibiotics directed against potential pathogenic intestinal flora [13]. All patients received amphotericin B lozenges and oral polymixin B; patients 3, 4, 8 and 9 also received intravenous temocillin. In case of an infection, defined as temperature $\geqslant$38.5°C (axillary) and clinical or bacteriological signs of infection, first-line antibiotic treatment consisted of cefuroxim and tobramycin. Nutritional support consisted of enteral tube feeding [14] and if necessary parental nutrition. Prophylactic

platelet transfusions were given at a platelet level below 15 × 10^9/l. A number of patients received cryopreserved autologous platelets [15], otherwise allogeneic single donor-platelets were used.

Toxicity

Physical examination and full blood cell counts were done daily, liver and renal functions three times a week. Neurological evaluation and electrocardiograms were performed weekly. Microscopic investigation of urine was done daily from day 1 to day 7, thereafter three times a week. Drug toxicity was graded according to WHO criteria (Table 2) [16]. Extramedullary grade 4 toxicity was considered to be dose-limiting.

Response

Complete response (CR) was defined as disappearance of all known tumor lesions and a return to normal of all relevant biochemical abnormalities over a period of at least 4 weeks. Partial response (PR) was defined as a decrease of more than 50% of the product of the largest perpendicular diameters of all measurable lesions for at least 4 weeks from the start of treatment. If in patients without an evaluable or measurable tumor lesion an established tumor marker (α-1-fetoprotein, human chorionic gonadotropin) decreased more than 90% this was also considered as a partial response [5]. Stable disease (SD) was defined as a less than 50% response without signs of progression. Progression was defined as an increase over 25% of a measurable lesion or appearance of new tumor lesions.

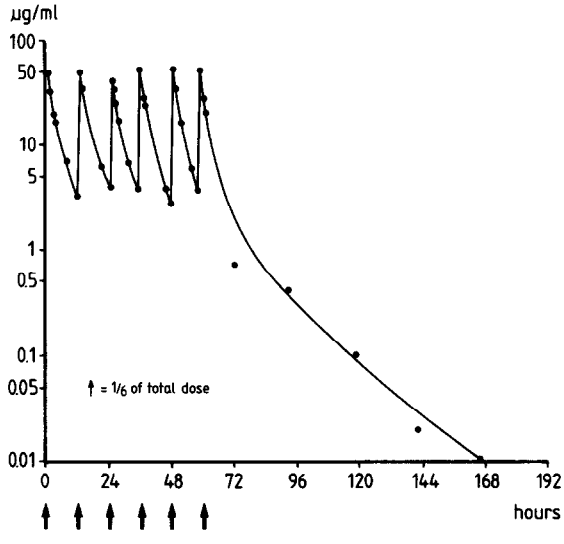

Fig. 1. Plasma levels of VP 16-213 after 1.5 g/m² VP 16-213 administration.

RESULTS

Pharmacokinetics

Low plasma levels of VP 16-213 could still be found on day 7 (day of marrow infusion). Detailed information on pharmacokinetic data will be published separately. A complete pharmacokinetic study is shown in one representative patient (Fig. 1).

Toxicity

During chemotherapy all patients experienced nausea and vomiting, grades 2–3. Some had moderate self-limiting diarrhea, grade 2. No bladder toxicity occurred. No hypertension, chills or fever were seen during the infusion of VP 16–213.

In one patient an anaphylactoid reaction occurred after infusion of about 80% of the stored bone marrow. Cardiac and pulmonary resuscitation was successful.

From day 4 until bone marrow recovery mucositis was severe and dose-limiting at 2.5 g/m² VP 16-213 (Table 3). Mucositis became evident at the 1.5 g/m² VP 16-213 dose level and therefore further dose escalations were modified to 2.0 and 2.5 g/m². Usually oropharyngeal erythema and soreness started on day 9 and recovered within 10 days. No other signs of digestive tract involvement were seen. Dermatitis was minimal (grade 0–2). There were no other important toxic side-effects. Neuropathy was not seen and no hemorrhagic complications occurred. Neither ECG abnormalities nor signs of cardiac failure were noted. All patients received platelet transfusions. Duration of granulocytopenia is

Table 2. Grading of observed toxic side-effects [16]

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Oral	no change	soreness/erythema	erythema, ulcers, can eat solids	ulcers, requires liquid diet only	alimentation not possible
Diarrhea	none	transient ≤2 days	tolerable, but >2 days	intolerable, requiring therapy	hemorrhagic dehydration
Cutaneous	no change	erythema	dry desquamation, vesculation, pruritis	moist desquamation, ulceration	exfoliative dermatitis, necrosis requiring surgical intervention

shown in Table 3. Fever due to infection was seen in ten patients (Table 4). In eight patients a causative microorganism was found, and two patients had clinical signs of infection. Two patients died during the cytopenic phase. In both patients high-grade fever developed during cytopenia, coinciding with erythematous dermatitis. In the following days fluid retention developed, probably due to a 'capillary leak syndrome'. Antibiotic treatment did not lead to improvement. Several cultures, including blood cultures, gave no support for an infectious origin of the fever. The patients gradually became confused and died on days 13 and 22 respectively. At autopsy no explanation for the fever was found.

Tumor response

In 9 patients response could be evaluated. In patients 1 and 2 before treatment macroscopic tumor was documented at laparotomy. After 10 months laparotomy in patient 1 did not reveal microscopic tumor. In patient 2 no clinical signs of tumor progression were seen, and a CT scan of the abdomen did not show any abnormalities. In patients 4, 9, 10, 11 and 12 α-1-fetoprotein levels decreased more than 90%. In patients 8 and 12 lung metastases showed a PR. In patient 5 mediastinal lymph nodes showed a PR on CT scan for more than 9 months without therapy. Hilar lymph nodes and primary tumor were unchanged in patient 6. In patient 3 routine investigations during follow-up did not reveal any signs of tumor progression anywhere for more than 5 months. At autopsy tumor was still present in patients 7 and 13.

DISCUSSION

In this study with high-dose cyclophosphamide and escalating doses of VP 16-213 the

Table 3. Toxicity

Patient	Mucositis grade	No. of platelet tranfusions	No. of days with granulocytes $\geq 0.5 \times 10^9/l$	First day post-transplant with granulocytes $\geq 0.5 \times 10^9/l$	No. of infused nucleated marrow cells $\times 10^8/l$
1	1	3	13	12	1.69
2	1	4	18	18	1.25
3	1	3	14	14	1.11
4	2	10	28	27	1.42
5	2	4	16	17	2.16
6	3	4	15	15	1.14
7	2	2*	*	*	1.63
8	4	4	12	10	0.92
9	3	4	17	16	1.3
10	3	3	13	12	1.2
11	2	4	22	20	1.5
12	4	5	13	13	1.4
13	4	5†	†	†	0.73

*Patient died on day 13, granulocytes 0, platelets $15 \times 10^9/l$.
†Patient died on day 22, granulocytes 0, platelets $15 \times 10^9/l$.

Table 4.

Patient	Infection days	Site of infection	Causative microorganism
1	0	-	-
2	10	esophagus	*Candida* spp.
3	3	skin at insertion of Hickman catheter	*Staph. epidermis*
4	3	skin	*Ps. fluorescens*
5	12	septicemia	*Strept. viridans*
6	6	skin of nose	Not found
7	0*	-	-
8	8	septicemia	*Strept. viridans*
9	5	sinusitis maxillaris	*Staph. epidermidis*
10	6	septicemia	*Strept. viridans* *Staph. epidermidis*
11	16	lung	*H. influenzae* *Strept. pneumoniae*
12	9	skin	*Serratia* spp.
13	0*	-	-

*Patient died, fever >40°C, no infection found.

dose limit of VP 16-213 appeared to be 2.5 g/m^2, due to mucositis of the oropharyngeal region. It is interesting to note that this mucositis was limited to the upper part of the digestive tract and that diarrhea was not seen.

The main and life-threatening toxicity in this study was infection. Its chief cause was the prolonged period of severe granulocytopenia. Earlier reinfusion of bone marrow was hampered by the slow elimination of VP 16-213. It is conceivable that isolation and gut sterilization would reduce the infection rate; however, its financial consequences would limit the applicability of this form of treatment to only a small percentage of all patients with solid tumors. The high infection rate could be influenced by the damage to the mucosal barrier of the oropharynx. In view of the limited toxicity to the lower digestive tract, it is tempting to speculate on a role of high saliva concentration of VP 16-213 as an explanation for the oropharyngeal mucositis [17].

Another major toxicity occurring in this study was the death of two older patients from a syndrome consisting of erythematous dermatitis, fever and fluid retention, finally leading to multiple organ failure. Although none of these other two signs occurred in the patients of this age group, we cannot recommend any of the tested dose regimens for patients of this age group. A syndrome comparable to this toxicity was recently described as 'the capillary leak syndrome' in mismatched as well as matched allogeneic bone marrow transplant patients [18]. Its incidence might be triggered by the radiomimetic effect of the alkylating agent, supposedly increased by age and the addition of VP 16-213.

Cyclophosphamide and VP 16-213 administered as single agents are not absolutely marrow ablative [9, 20]. Although no increase in the time until recovery was noted at the escalating dose levels, it is impossible to conclude whether the combination of the drugs necessitates the use of autologous bone marrow transplantation, as has been done in this study. We can conclude that cyclophosphamide at 7 g/m^2 and VP 16-213 at 1.5 g/m^2 can be given without major extramedullary toxicity when combined with ABMT. However, infectious complications are still an important problem and perhaps an age limit should be introduced because of the above-mentioned 'capillary leak syndrome'.

The response rate and duration, however, make this approach worthwhile for study in the tumor types treated in this report. Especially for SCLC, this combination of cyclophosphamide and VP 16-213 may be important for late intensification studies. Bone marrow harvesting after remission induction with standard dose therapy results in a minimal risk of bone marrow contamination, while marrow reserve as measured by granulocyte colony-forming units is still sufficient [21]. VP 16-213 in this combination can be administered at a dose of 1.5 g/m^2 without major extramedullary toxicity. At this dose a sanctuary site, the central nervous system (CNS), can be reached in apparently effective concentrations [22]. This can be important for prophylaxis of CNS metastases. The two patients with ovarian cancer have clearly benefited from this treatment and more patients with minimal residual disease will be treated with this regimen. Furthermore, the addition of drugs with dose-limiting toxicity other than encountered in this regimen, for instance *cis*-platinum, might increase the therapeutic potential.

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