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Original article

Multiple cycles of high-dose doxorubicin and cyclophosphamide with G-CSF mobilized peripheral blood progenitor cell support in patients with metastatic breast cancer

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Summary

Background: In a previous study we applied doxorubicin and cyclophosphamide in a dose-intensive regimen with GM-CSF to patients with metastatic breast cancer (MBC). That treatment failed to prolong the remission duration compared to conventional-dose chemotherapy. In the present study we escalated the dosages of the same agents to: 1) determine the maximum tolerated dosages (MTD) when given for three cycles with G-CSF mobilised peripheral blood progenitor cell (PBPC) reinfusion and 2) evaluate the antitumour effect of this regimen.

Patients and methods: For mobilisation of PBPC, G-CSF 15 μg/kg/day was given subcutaneously (s.c.), and in subsequent cohorts leucapheresis was started on days 3, 4 or 6. The intention was to treat MBC patients with three cycles of doxorubicin and cyclophosphamide at a starting dose of doxorubicin 90 mg/m^2 and cyclophosphamide 1000 mg/m^2. Dosages were then escalated in subsequent cohorts of at least three patients. In case of dose-limiting mucositis, only the dose of cyclophosphamide was escalated in the next cohort.

Results: Twenty-one patients entered this protocol, of which 18 patients received high-dose chemotherapy. The mobilisation of PBPC using G-CSF only was sufficient for three cycles of high-dose chemotherapy in 10 of 21 (47%) patients. Mucositis precluded dose escalation of doxorubicin beyond 110 mg/m^2. The MTD in this combination was 110 mg/m^2 for doxorubicin, and 4 g/m^2 for cyclophosphamide, with haemorrhagic cystitis being the dose-limiting toxicity. The overall response rate was 78% (95% confidence interval (95% CI): 57%–97%), with 22% (95% CI: 3%–41%) complete responses.

Conclusion: The MTD of this three cycle high-dose regimen was doxorubicin 110 mg/m^2 and cyclophosphamide 4 g/m^2 with mucositis and cystitis being dose-limiting toxicities. Although the primary aim was not the evaluation of antitumour effect, this high-dose regimen does not appear to provide an improvement of treatment results in comparison with our previous study with the same drugs at moderately high-dosages without stem cell support.

Key words: breast cancer, high-dose chemotherapy

Introduction

High-dose chemotherapy followed by either autologous bone marrow transplantation (ABMT) or the reinfusion of peripheral blood progenitor cells (PBPC) is being increasingly used in an attempt to improve survival in metastatic breast cancer (MBC) patients. The rationale for this approach is found in the steep dose-response curve for several cytotoxic agents in breast cancer [1, 2].

In a previous study we treated MBC patients with a median of five cycles of moderately high-dose doxorubicin and cyclophosphamide at a 21-day interval [3], with the use of GM-CSF to support haematologic recovery [4]. This regimen yielded a high response rate of 82% (95% CI: 69%–97%), but no apparent increase in disease free survival (DFS) or overall survival (OS) compared with conventional chemotherapy [3]. In the present study we further escalated the dosages of the same cytotoxic agents with the use of PBPC and G-CSF to investigate whether this would improve the results.

Generally, one cycle of high-dose chemotherapy in MBC patients has been applied to patients responding to conventional-dose chemotherapy [5, 6]. Tumour cells may, however, have already developed resistance after such a short-term exposure to drugs [7]. It is, therefore, probably more effective to administer high-dose chemotherapy in previously untreated MBC patients. Cancers which can be cured by chemotherapy, such as Hodgkins's disease, malignant lymphoma and germ cell cancer, all require delivery of multiple cycles at timely intervals of an effective regimen [8]. Considering these points, we investigated the feasibility of three cycles of high-dose doxorubicin and cyclophosphamide with PBPC reinfusion in previously untreated MBC patients.

For mobilisation of PBPC only G-CSF was used. With this strategy cancer cells will not be exposed to any chemotherapy prior to the challenge with the high-dose, thus limiting the chance of the emergence of drug resistance [7].

Cyclophosphamide is commonly used in high-dose

transplantation regimens [5, 6]. It is active against non-proliferating cells while relatively sparing haematopoietic stem cells [9]. Doxorubicin is not often used in high-dose regimens and is probably more toxic than cyclophosphamide to stem cells. For this reason we examined the toxicity of several doxorubicin concentrations on the Colony-Forming-Unit (CFU) capacity of cells harvested by leucapheresis, to determine at which moment after doxorubicin administration the stem cells can be reinfused savely.

In the present trial we studied: 1) the feasibility of mobilising PBPC with G-CSF alone to support three cycles of the high-dose regimen, 2) established the maximum tolerated dosages (MTD) of three cycles of doxorubicin and cyclophosphamide with PBPC reinfusion, 3) determined the optimal time of reinfusion of PBPC, and 4) studied the antitumour effect of this regimen.

Patients and methods

Patient selection

Patients were eligible if they had MBC, with at least one measurable or evaluable lesion according to World Health Organisation (WHO) criteria [15], and were without brain metastasis and/or meningeal disease. Age below 55 years, and a WHO performance status $\leqslant 2$ were additional entry criteria. Prior chemotherapy was not allowed, except for non-anthracycline containing adjuvant chemotherapy at least one year before study entry. Pre-treatment assessment included a medical history and physical examination, chest X-ray, bone scan, abdominal CT scan, ECG, left ventricular ejection fraction (LVEF) and baseline laboratory investigations. Adequate bone marrow function (white blood cell count (WBC) $\geqslant 4.0 \times 10^9$/l, platelets $\geqslant 100 \times 10^9$/l), renal function (creatinine clearance $\geqslant 60$ ml/min) and hepatic function (bilirubin $\leqslant 25$ µm/l, AF, SGOT and SGPT $\leqslant 2\times$ normal value) were required. Patients with a LVEF $\leqslant 50\%$ were excluded. While on treatment, all patients had a medical history, physical examination, baseline laboratory tests, chest X-ray, ECG and LVEF before each cycle. Full blood counts were performed three times a week and biochemical analysis weekly. Evaluation of response was carried out after every cycle. Response and toxicity were recorded according to WHO criteria [10]. After completion of the chemotherapy, patients who responded were additionally treated with surgery and/or radiotherapy when feasible. Written informed consent was required and the protocol was approved by the ethical and scientific committees of the hospital.

Peripheral blood progenitor cell mobilisation and harvest

PBPC were collected after mobilisation with G-CSF 15 µg/kg subcutaneously (s.c.) once daily until the last day of leucapheresis. In the first six patients leucapheresis was started on day 6 of the administration of G-CSF. Because of insufficient harvest results in these patients it was decided to start at day 3 in the next patients. Harvest on day 3 was, however, never substantial, and we therefore decided to start on day 4 after another five patients. Minimum requirements for harvest included at least 6×10^6 CD34+ cells/kg, and at least 30×10^4 CFU-GM/kg. If these requirements were not met, additional mobilisation and harvest of progenitor cells were performed after the first cycle of chemotherapy. G-CSF 5 µg/kg/day s.c. was started 48 hours after chemotherapy and leucapheresis was started when the WBC rose above 3×10^9/l. The leucaphereses were carried out using a continuous-flow blood cell separator (Fenwal CS3000, Baxter Deutschland GmbH, Germany). The blood volume processed in each session was 10 liters. The cell yields were counted, the number of CD34+ cells were determined by flow cytometry, and the number of CFU-GM was measured. The cells were cryopreserved in plasma and 10% dimethyl sulfoxide (DMSO) at a maximal cell concentration of 200×10^6 mononuclear cells (MNC)/ml. For cryopreservation, the cell suspensions were frozen at a controlled rate using a Kryo10 (Cryotech, Schagen, the Netherlands). Frozen cells were stored in liquid nitrogen until reinfusion.

High-dose chemotherapy

Chemotherapy consisted of three cycles with starting dosages of doxorubicin 90 mg/m^2 and cyclophosphamide 1000 mg/m^2. In subsequent cohorts of at least three patients the dose of doxorubicin was increased by 10 mg/m^2 and the dose of cyclophosphamide by 1000 or 2000 mg/m^2 (Table 2). Chemotherapy was started when results of CFU-GM cultures were available. Cyclophosphamide was administered in 0.5 l of 0.9% normal saline in a two-hour infusion combined with mesna at 50% of the cyclophosphamide dose and followed by 4 l of 0.9% normal saline with mesna at the same dose as cyclophosphamide, in 24 hours. At the fifth dose level cyclophosphamide was administered as a 24-hour continuous infusion in an attempt to decrease toxicity because of lower peak levels [11]. Doxorubicin was administered in 0.5 l of 0.9% normal saline in a six hours infusion and started immediately after cyclophosphamide. Chemotherapy was administered via a central venous catheter. G-CSF 5 µg/kg/day was started in all patients 48 hours after chemotherapy until absolute neutrophil counts (ANC) were $\geqslant 0.5 \times 10^9$/l. When indicated, PBPC were reinfused 48 hours after the end of chemotherapy. Prophylactic antibiotic and antifungal therapy were given until ANC $\geqslant 0.5 \times 10^9$/l and consisted of oral ciprofloxacin (500 mg b.i.d.), fluconazole (50 mg) and amphotericin nose spray. For streptococcal prophylaxis roxitromycin 150 mg b.i.d. was administered. Throat, urine and faeces cultures were taken twice a week. In case of a rise in temperature above 38.5 °C, neutropenic patients were empirically treated with broad-spectrum antibiotics. Transfusions of leucocyte-free red blood cells were given when the haemoglobin level felt below 6.0 mmol/l, and six donor units of platelets were administered when platelets were $\leqslant 10 \times 10^9$/l, or in case of bleeding. Blood products were irradiated for patients requiring PBPC reinfusion, and these patients remained in the hospital until recovery of ANC to $\geqslant 0.5 \times 10^9$/l. Courses were planned at three-week intervals. Complete bone marrow recovery was not required if PBPC were to be reinfused, but any mucositis and any infection needed to be controlled. First the MTD without PBPC was established. In case of dose limiting mucositis, only the dose of cyclophosphamide was escalated in the next cohort. When the MTD for haematologic toxicity was reached, PBPC were reinfused in the next cohort of patients until non-haematologic toxicity precluded further dose escalation. The MTD was defined as the dose at which any of the following events occurred in two out of three patients in any of the three cycles; WHO grade 4 thrombocytopenia requiring platelet transfusion, ANC $\leqslant 0.5 \times 10^9$/l for more than seven days, ANC $\leqslant 0.1 \times 10^9$/l for more than three days, episodes of febrile neutropenia (temperature $\geqslant 38.5$ °C and ANC $\leqslant 0.5 \times 10^9$/l requiring i.v. antibiotics), WHO grade $\geqslant 3$ mucositis for more than seven days, any WHO grade $\geqslant 3$ of non-haematological toxicity, excluding alopecia, and vomiting grade 3.

Toxicity of doxorubicin on CFU-forming cells

Doxorubicin serum concentrations were determined at 24 and 48 hours after doxorubicin infusion in four patients. From these data the doxorubicin concentration at different time points was calculated for these patients, according to the known clearance of doxorubicin from the blood [12]. Samples from the leucaphereses harvest of these patients were used to determine the cytotoxicity of different doxorubicin concentrations on CFU-forming cells. To test this cytotoxicity, the *in vivo* situation was mimicked as follows: from the leucapheresis harvests cells were plated in flat bottomed wells (5×10^6 cells/well in 5 ml RPMI medium containing 20% fetal calf serum (FCS)). Doxorubicin was added in a concentration range from 0–200 nmol/l (eight

Table 1. Pre-treatment characteristics of the patients.

Totals	
Patients	18
Age, median (range)	48 (28–55)
Receptor status	
Unknown	2
ER+, PR+	2
ER+, PR−	1
ER−, PR−	13
ER−, PR+	0
Prior adjuvant chemotherapy[a]	4
Prior adjuvant hormonal therapy	1
Time to relapse, median (range)	27 (12–48)
Prior therapy at relapse	
Hormonal therapy	3
Radiotherapy	3
None	12
No. of involved sites	
1	6
2	8
3	2
4	2
Actual disease site	
Breast	6
Lymph nodes	7
Bone	8
Lung	4
Liver	6
Skin	3
Ovary	1
Peritoneum	1

[a] All patients received cyclophosphamide, 5-fluorouracil and methotrexate.
Abbreviations: ER – estrogen receptor; PR – progesteron receptor.

concentrations) to the different wells. In each well, the doxorubicin concentration was diluted with RPMI and 20% FCS each 15 hours, according to the calculated half-time curve of doxorubicin. After eight days cells were harvested, washed, counted and 30,000 cells were plated in 300 µl CFU-medium in a 24-well plate. The number of CFUs were scored after 12 days. Each initial doxorubicin concentration thus corresponds with a certain percentage of CFU-capacity left. Since plasma doxorubicin concentrations can be calculated at each time point after doxorubicin infusion, this makes it possible to establish how much CFU-capacity would have been left if the stem cells would have been reinfused at these different time points.

Results

From March 1994 until December 1995, 21 patients were entered into this protocol. Results of PBPC mobilisation and harvest were available for three patients who were not treated with the high-dose chemotherapy after leucapheresis for the following reasons: one patient had brain metastases, another patient with liver metastases developed a tenfold raise of serum bilirubin shortly after the mobilisation with G-CSF which prohibited treatment with doxorubicin, and the third patient developed rapidly progressive renal insufficiency. The characteristics of the 18 patients treated with high-dose chemotherapy are summarised in Table 1. The dose escalation scheme and the number of patients included at each dose level are depicted in Table 2. At the first dose level 4 patients were entered because not all toxicity data were available when the fourth patient entered the protocol. In dose level 4 five patients were treated; one patient discontinued treatment after one cycle because of toxicity, whilst toxicity data were still incomplete when the fifth patient was entered. Median treatment interval between different courses was 21 days (range 19–30 days).

Peripheral blood progenitor cell mobilisation and harvest

In the 21 patients a median of 6.15×10^6 CD34+ cells/kg were harvested (range $1.95–17.40 \times 10^6$), with a median of 35×10^4 CFU-GM/kg (range $10–90 \times 10^4$/kg) after mobilisation with G-CSF. A median of three (range 1–5) leucaphereses were required. In 10 patients (47%), the pre-determined number of CD34+ cells for three reinfusions were harvested ($\geqslant 6 \times 10^6$ CD34+ cells/kg), while in all patients sufficient CD34+ cells were obtained for at least one reinfusion. The optimal day of harvesting appeared to be day 5 after start of G-CSF administration (Figure 1). When CD34+ yield was insufficient for three reinfusions, leucaphereses after the first cycle of chemotherapy was always successful in harvesting enough CD34+ cells for subsequent reinfusions, with a median number of CD34+ cells of 7.2×10^6/kg (range $2.0–18.7 \times 10^6$) in one or two sessions.

Haematologic toxicity

Haematologic toxicity observed is listed in Tables 3 and 4. Neutropenia grade 4 occurred in almost every cycle at dose levels 1 and 2, but because of the short duration it was not dose-limiting. Platelet transfusions were not indicated at dose level 1. At dose level 2, all cycles except two were accompanied by grade 4 thrombocytopenia, and all patients needed platelet transfusions in two or three cycles, indicating the MTD for haematologic toxicity. At dose levels 3–5 platelet transfusions had to be given once or twice in every cycle. Red blood cell transfusions were necessary in 5 of 12 cycles at dose level 1, eight of nine cycles at dose level 2, and once or twice during every cycle at dose levels 3–5.

Neutropenic fever requiring hospital admission for intravenous antibiotic treatment did not occur at the first dose level, but at dose level 2 every patient was admitted for neutropenic fever. Two patients were admitted in every cycle and one patient in one cycle, again indicating the MTD for haematologic toxicity at dose level 2. No positive blood cultures were obtained in these patients. Neutropenic fever accompanied 10 of 27 cycles at dose levels 3–5, and positive blood cultures were found in six cycles. Micro-organisms isolated were *Staphylococcus aureus* and *Staphylococcus epidermidis*. Herpes zoster was observed in one patient at dose level 5.

Time to neutrophil recovery ($\geqslant 0.5 \times 10^9$/l), and

Table 2. Dose-escalation scheme, number of patients included and response to chemotherapy.

Level	Dox mg/m^2	Cyclo mg/m^2	No. of patients	No. of cycles	CR	PR	SD	PD
1	90	1000	4	12	–	3	1	–
2	100	2000	3	9	2	1	–	–
3	110	3000	3	9	1	2	–	–
4	120	4000	5	13	1	2	2	–
5	110	6000	3	5	–	2	–	1

Abbreviations: Dox – doxorubicin; Cyclo – cyclophoshamide; CR – complete remission; PR – partial remission; SD – stable disease; PD – progressive disease.

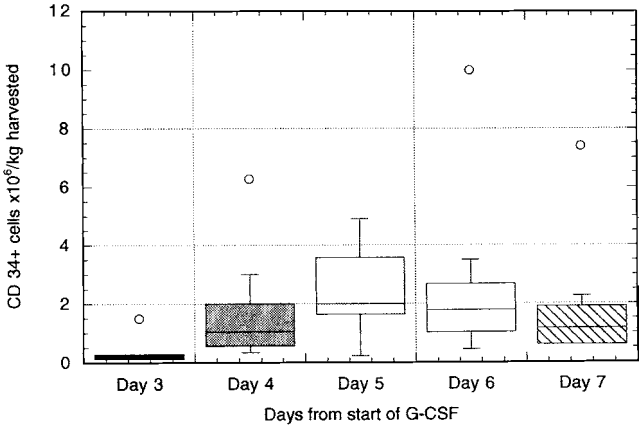

Figure 1. CD34+ harvest at day 3–7 after mobilisation with G-CSF only. Box shows 75th percentile, arrow bars show 90th percentile and o shows exceptional values.

platelet recovery ($\geqslant 20 \times 10^9$/l) was quite similar at the various dose levels. The median time for recovery of neutrophils was 13 days (range 11–16) after start of chemotherapy, and median time to platelet recovery was 14 days (range 11–16) in the first cycle and 18 days (range 14–22) in the third cycle.

Non-haematologic toxicity

The most important non-haematologic toxicities were mucositis (Table 5) and haemorrhagic cystitis. Dose-limiting mucositis occurred at dose level 4; in the third cycle one patient experienced grade 3 mucositis for more than seven days and grade 4 mucositis occurred in three patients for 4–6 days. The study was continued with the reduced dose of 110 mg/m^2 doxorubicin while cyclophosphamide was escalated further.

Haemorrhagic cystitis was observed in one patient at dose level 4 after the first cycle of chemotherapy, which was the reason to discontinue the therapy. Other patients at this dose level did not experience haemorrhagic cystitis. At dose level 5, the protocol was amended and cyclophosphamide was administered in a continuous infusion for 24 hours to try to reduce toxicity. Two patients treated at this dose level, however, also experienced haemorrhagic cystitis; one during the first cycle and the other during the second cycle. In both patients the cystitis resolved after 2–3 weeks, and in subsequent cycles cyclophosphamide was omitted in these patients. Nausea and vomiting were nearly always manageable with ondansetron and dexamethasone. Only one patient at dose level 5 experienced grade 4 vomiting in cycles and cycle 2. General weakness was present in every cycle and was cumulative. All patients, except one, recovered to WHO performance status 1 or 2 by day 21. The exception was the third patient at dose level 5 who did not recover until six weeks after the second cycle of high-dose chemotherapy. For this reason she did not receive a third cycle. Cardiotoxicity did not occur. Only one patient experienced a transient decline in LVEF which occurred after the first cycle at dose level 3. A cardiac biopsy was performed which did not show abnormalities in the myocardium, and treatment was continued. After the second and third cycle LVEF remained normal.

Table 3. Absolute neutrophil count (ANC) ($\times 10^9$/l) median (range) by dose level and cycle.

Dose level	Cycle 1		Cycle 2		Cycle 3	
	ANC	Days $\leqslant 0.5$	ANC	Days $\leqslant 0.5$	ANC	Days $\leqslant 0.5$
1.	0.3 (0.2–0.3)	4 (3–5)	0.3 (0.2–0.5)	3 (2–4)	0.3 (0.2–0.3)	3 (2–5)
2.	0.1 (0.1–0.2)	5 (3–7)	0.1 (0.1–0.2)	5 (2–6)	0.1 (0.1–0.2)	5 (4–5)
3.	0.1 (0.1–0.2)	5 (4–7)	$\leqslant 0.1$ –	5 (5–6)	$\leqslant 0.1$ –	7 (7–8)
4.	$\leqslant 0.1$ –	6 (6–7)	$\leqslant 0.1$ –	7 (6–7)	$\leqslant 0.1$ –	8 (7–9)
5.	$\leqslant 0.1$ –	5 (5–7)	$\leqslant 0.1$, $\leqslant 0.1$	6, 7	NA	–

Abbreviation: NA – not available.

Table 4. Platelet count (PLT)($\times$ 10^9/l) median (range) by dose level and cycle.

Dose level	Cycle 1		Cycle 2		Cycle 3	
	PLT	Days $\leqslant 20$	PLT	Days $\leqslant 20$	PLT	Days $\leqslant 20$
1.	70 (60–100)	–	62 (50–85)	–	53 (48–58)	–
2.	15 (2–44)	2 (2–3)	14 (11–48)	2 (2–4)	12 (7–25)	3 (3–6)
3.	7 (5–7)	4 (4–6)	6 (5–10)	4 (4–7)	8 (7–10)	8 (7–12)
4.	8 (5–16)	4 (3–6)	8 (1–9)	5 (4–8)	8 (1–9)	8 (7–11)
5.	8 (7–9)	4 (3–5)	8, 8	3, 5	NA	NA

Abbreviation: NA – not available.

Table 5. Mucositis (grade, duration) median (range) by dose level and cycle.

Dose level	Cycle 1		Cycle 2		Cycle 3	
	Grade	Days $\geqslant$ gr 3	Grade	Days $\geqslant$ gr 3	Grade	Days $\geqslant$ gr 3
1.	1 (0–1)	–	1 (0–1)	–	1 (0–2)	–
2.	1 (0–2)	–	2 (1–2)	–	2 (2–3)	3
3.	2 (2–3)	3	2 (2–3)	3	3 (3–3)	4, 4, 5
4.	3 (2–3)	3, 3, 3	4 (2–4)	4, 4, 6	4 (3–4)	4, 4, 6, 7
5.	2 (1–3)	4	(2, 4)	5	–	–

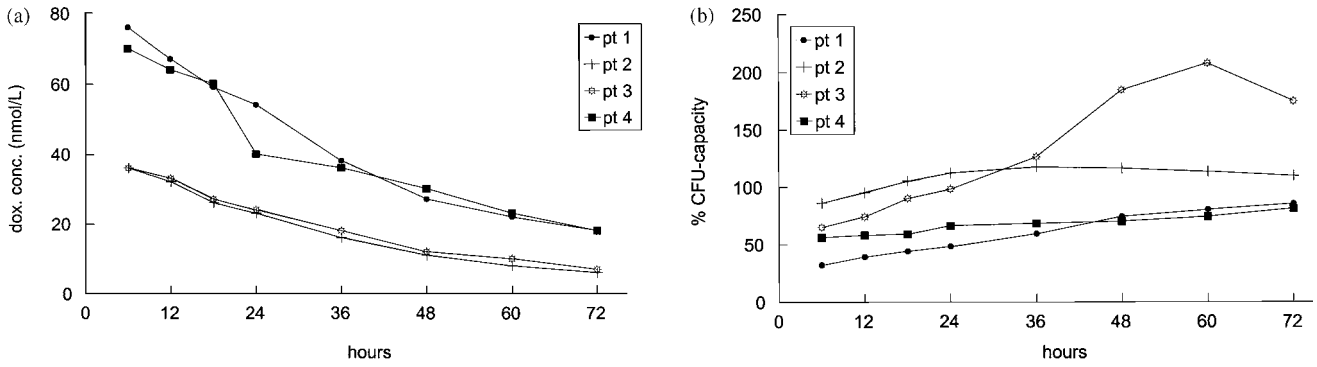

Figure 2. (a) Concentration *versus* time curve of doxorubicin in four patients. (b) Calculated CFU-capacity (%) of PBPC after different time points of doxorubicin infusion in four patients.

Because it was impossible to administer three cycles of doxorubicin 110 mg/^{2} and cyclophosphamide 6000 mg/m^2 the MTD for the combination of these drugs with this schedule was defined as doxorubicin 110 mg/m^2 and cyclophosphamide 4000 mg/m^2.

Response

Fourteen of the 18 patients had a response to the chemotherapy, the overall response rate was 78% (95% CI: 57%–97%), and the complete response rate (CR) (22%; 95% CI: 3%–41%). There seemed to be no trend towards more complete remissions with increasing dosages (Table 2).

Toxicity of doxorubicin on CFU forming cells

The calculated doxorubicin concentrations at different time points are shown in Figure 2a for the four patients. Figure 2b shows the remaining CFU-capacity of the cells which are harvested by leucaphereses at the different time points after doxorubicin infusion. The results show a great intra-patient variability. For one patient the remaining CFU-capacity of the leucaphereses harvest was already 70% six hours after doxorubicin infusion, while for two other patients this CFU-capacity was only present after 48 hours.

Discussion

The primary aim of this study was to establish the MTD for the combination of doxorubicin and cyclophosphamide with reinfusion of PBPC. At dose level 2 we observed the MTD for haematologic tixicity, as defined in the methods section. One can argue whether PBPC were really necessary. For cyclophosphamide it is known that it is possible to give 7000 mg/m^2 without PBPC, and the quite similar time to recovery in the different dose levels suggest that an important role was still being played by endogenous reconstitution. However, because we wanted to give three cycles of closely timed high-dose chemotherapy, we defined quite strict criteria for the haematologic MTD. The progressively delayed recovery observed after subsequent cycles, especially for the platelets, might probably be ascribed to the alterations in the microenvironment of bone marrow, which have been suggested to occur after high-dose chemotherapy [13, 14].

Development of mucositis precluded escalation of the dose of doxorubicin above 110 mg/m^2. This toxicity appeared to be cumulative; because no patient, even at a dose of 120 mg/m^2, experienced dose limiting mucositis in cycle one, but in the third cycle all patients treated with doxorubicin 120 mg/m^2 developed mucositis. The feasibility of administering 165 mg/m^2 doxorubicin in combination with cyclophosphamide and etoposide has been reported [15], but these investigators applied only one cycle.

The non-haematologic dose-limiting toxicity of this regimen was chemical cystitis. An attempt to decrease the risk of haemorrhagic cystitis by lenghtening the duration of cyclophosphamide infusion was not succesfull. Our high incidence of haemorrhagic cycstitis is difficult to explain. The administration of 7 g/m^2 cyclophosphamide in five divided dosages in a one-hour infusion given in a 13-hour period has been reported without any cystitis [16, 17]. It is possible that this complication is schedule dependent. The pharmacokinetics of cyclophosphamide are dose and time dependent and prolonged infusions show a gradual change in clearance of the drug following a latency period [18]. This nonlinear elimination exhibited by some patients receiving high-dose cyclophosphamide may have increased the risk of toxicity. This aspect of the pharmacology needs further investigation. Another possibility is that the high-dose of doxorubicin enhances the toxicity of cyclophosphamide to the urothelium. Other toxicities of this regimen were manageable.

A secondary aim of this study was to investigate whether sufficient PBPC for 3 high-dose chemotherapy cycles could be harvested using G-CSF alone. The minimum number of cells required to ensure rapid reconstitution remains controversial, but seems to be in the range of 10×10^4 CFU-GM/kg and $2–3 \times 10^6$ CD34+ cells/kg [19]. In the present study, mobilisation with only G-CSF yielded a sufficient number of PBPC for three

cycles of high-dose chemotherapy in only 47% of patients. Sufficient cells for one cycle of high-dose chemotherapy were harvested in all patients. Other groups have attempted to mobilize PBPC in previously untreated MBC patients with G-CSF alone. In one of these studies [20] similar numbers of PBPC were obtained and in two [21, 22] 3–4 times more could be harvested. The reasons for these differences are unclear although technical differences in culture and flow-cytometric techniques might play a role.

Toxicity of doxorubicin to CD34+ cells in our *in vitro* model, which only partly reflects the *in vivo* situation, showed an intra-patient variation, but indicated that $\geqslant 70\%$ of CFU capacity was present after 48 hours of doxorubicin infusion in all patients. In some patients, however, it seems safe to reinfuse stem cells at an earlier time point, but this needs to be studied for individual patients. In our multiple cycle regimen we observed a more delayed recovery of granulocytes and especially of platelets after each cycle, thus earlier reinfusion might be of some benefit allowing for earlier recovery of haematopoiesis.

The overall response rate of this regimen was 78% (95% CI: 57%–97%) and 22% (95% CI: 3%–41%) of patients achieved a complete response. These results are comparable to our previous study with doxorubicin 90 mg/m^2 and cyclophosphamide 1000 mg/m^2 with GM-CSF given for a median of 5 cycles, where we observed a response rate of 82% (95% CI: 69%–97%), with 32% (95% CI: 14%–50%) complete remissions [3]. Although our study was a dose-finding study, the data do not encourage us to use this regimen in a randomised study comparing it with conventional-dose doxorubicin and cyclophosphamide in advanced breast cancer.

In conclusion, the mobilisation of PBPC with G-CSF alone is feasible for one cycle of high-dose chemotherapy but other mobilisation strategies are required to allow multiple cycles to be given. In addition, the administration of multiple cycles of high-dose chemotherapy is feasible but the escalation of the doses of doxorubicin and cyclophosphamide resulted in severe mucositis and cystitis and apparently failed to improve complete response rate in comparison with our earlier study. Potentially more effective regimens containing taxanes are currently under investigation.

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