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Original article

High-dose thiotepa and melphalan with hemopoietic progenitor support following induction therapy with epirubicin–paclitaxel-containing regimens in metastatic breast cancer (MBC)

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Summary

Background Preliminary data from phase III randomized studies have failed to show benefit of HDC given as consolidation after anthracycline and alkylating-based chemotherapy in metastatic breast cancer (MBC). Moderate activity of induction regimens and selection of chemoresistant clones are among the possible reasons for these disappointing results. We therefore have designed a phase II study where high-dose alkylating agents are given as consolidation after an induction treatment including the most active agents (epirubicin and paclitaxel) without alkylating agents.

Patients and methods Patients with MBC not previously treated with chemotherapy for metastatic disease were eligible. After six courses of epirubicin–paclitaxel ± gemcitabine patients received a course of thiotepa 600 mg/m^2 + melphalan 160 mg/m^2 with hemopoietic support. Pharmacokinetic parameters of thiotepa and melphalan were measured and related to treatment outcomes. The L-VEF of the patients was monitored before and after treatment.

Results Forty-eight patients have been treated. Before HDC 14 patients were in CR, and 34 in PR. A median of 6.92 × 10^6 (range 1.53–16.6) CD34+ cells/kg were reinfused after HDC. Median days (range) to neutrophils > 0.5 × 10^9/l and platelets > 20,000 × 10^9/l were 9.5 (9–33) and 10 days (9–32), respectively. Symptomatic CHF was observed in two patients (4.1%). C_{max} and AUC of thiotepa showed a linear relationship with time to progression (TTP) and overall survival (OS). r^2 = 0.6. After HDC the conversion rate from PR to CR was 44.1%. At five years progression-free and overall survival rates are 37.5% and 65%, respectively. A treatment-related death was observed.

Conclusions High-dose thiotepa and melphalan after an epirubicin–paclitaxel-containing treatment is feasible, devoid of significant cardiotoxicity and very active. Pharmacokinetic parameters of high-dose thiotepa might be linked to treatment outcome.

Key words high-dose chemotherapy, metastatic breast cancer, paclitaxel

Introduction

High-dose chemotherapy (HDC) with autologous hemopoietic support has been increasingly used in MBC responsive to standard chemotherapy, however, despite preclinical data and phase II studies, preliminary data from phase III randomized studies have failed to show benefit of HDC in chemoresponsive MBC [1, 2]. Most of the randomized trials have tested the strategy of induction standard dose chemotherapy for four to six courses followed by late intensification with a single course of HDC. High-dose regimens usually include alkylating agents because of their steep dose-response curve, non-cycle specificity and broad clinical activity [3, 4]. Melphalan and thiotepa have been used at high doses as single agents or in sequential combination in chemoresponsive MBC with a complete remission rate ranging between 40% to 90%. At Memorial Sloan-Kettering Cancer Center the sequential use of the two drugs resulted in a higher complete response rate compared to that observed in a prior study with two courses of thiotepa [5]. Data from Ayash et al. [6] and Bitran et al. [7] support the hypotesis that high-dose melphalan might be not cross-resistant with high-dose thiotepa.

Recently, a number of new cytotoxic agents have become available for clinical use and some of these (i.e., taxanes, gemcitabine) have shown interesting activity in MBC. Several studies have evaluated the activity and the optimal schedule of these new drugs combined with anthracycline. Our group has shown that the combination of epirubicin plus paclitaxel (ET) induces a response rate of 84% with 19% CRs while gemcitabine + ET (GET) produces a response rate of 92% (CR 31%), moreover both regimens + G-CSF are able to effectively mobilize peripheral blood progenitors [8–10].

On these premises we have designed a phase II study

of high-dose thiotepa and melphalan as consolidation treatment following induction with an ET-containing regimen. The primary end-points of this study were: 1) to evaluate the toxicity, particularly cardiotoxicity, of high-dose chemotherapy following a treatment including paclitaxel + anthracycline; 2) to assess the activity of high-dose thiotepa + melphalan in chemoresponsive patients not exposed to alkylating agents in the induction phase. Secondary end points were progression-free and overall survival and pharmacokinetic parameters of high-dose thiotepa followed by high-dose melphalan. So far the drug disposition of high-dose alkylating agents has been evaluated following either high-dose thiotepa or high-dose melphalan as single agents [11–13].

Patients and methods

Eligibility

Women < 60 years old, performance status 0–1 (ECOG scale) with hystologically confirmed metastatic breast cancer in complete or partial remission following induction chemotherapy with ET or GET were eligible for HDC consolidation treatment with peripheral blood progenitor cell (PBPC) support Other eligibility criteria included left ventricular ejection fraction (L-VEF) > 50%, as determined by bidimensional echocardiography, no history of cardiac abnormality, negative HBsAg, HCV and HIV Prior hormonal therapy and/or prior adjuvant chemotherapy were permitted Patients with bone disease only were excluded The presence of morphologically or immunohistochemically positive bone marrow was not an exclusion criteria The patients had to sign an informed consent and the Institutional Ethics Committee of Pisa University Hospital approved the study

Treatment plan: Induction phase

In the induction phase the patients received epirubicin 90 mg/m^2 plus paclitaxel 200 mg/m^2 administered as three-hour infusion on day 1 every 21 days (ET) or ET plus gemcitabine 1 g/m^2 on day 1 and 4 every 21 days (GET)

Peripheral blood progenitor cells (PBPC) were mobilized after the third course of chemotherapy with recombinant human granulocyte colony stimulating factor (rhG-CSF) 5 μg/kg/day s c starting 24 hours after the end of chemotherapy

When the absolute count of CD34+ cells exceeded 50/μl, leukapheresis procedure were performed on the out-patient basis using a Fenwall CS 3000 cell separator (Baxter, Chicago, Illinois) or COBE Spectra Eight to ten liters of whole blood were processed per each procedure using continuous flow centrifugation at a flow rate of 50 ml/min

Treatment plan: High-dose regimen

The HDC regimen included thiotepa 600 mg/m^2 (day −3) and melphalan 160 mg/m^2 (day −1) 48 hours apart Thiotepa was given divided in three equal doses as one-hour infusion with a one-hour interval between each dose, melphalan was divided in three equal doses as i v bolus two hours apart each. During the three days of treatment, the patients were hydrated with 3000 ml/m^2 of 0 9% NaCl saline solution and received antiemetic therapy consisting of dexamethasone 20 mg daily and ondasentron 48 mg daily. Autologous PBPC were reinfused 24 hours after the end of chemotherapy, at the time of reinfusion frozen PBPC were thawed rapidly in a 37 °C warm bath and reinfused through venous central catheter Following PBPC reinfusion, rhG-CSF 5 μg/kg/day was administered until WBC > 1000/ml for three consecutive days Patients were housed in a single room and given oral prophylaxis with ciprofloxacin and fluconazole and standard mouth care, patients with Herpes simplex positive titer were given acyclovir 500 mg/i v every 12 hours. In case of febrile neutropenia ciprofloxacin was discontinued and a combination of i v ceftazidime + amikacin was administered Total parenteral nutrition was given in case of severe mucositis impairing food intake Patients were transfused with irradiated (25 Gy) leukocyte-free packed RBCs and single-donor platelets to maintain hemoglobin values above 9 g/dl and platelet counts above 15,000/μl Patients were discharged when the absolute neutrophil count was > 500/μl, and they were able of oral feeding intake and did not require platelet support daily

Treatment evaluation

A complete restaging of disease extent was performed with appropriate imaging techniques two weeks before and one month after HDC Toxicities and responses were reported according to WHO criteria Overall survival and progression-free survival were calculated from the day of the first course of chemotherapy

Pharmacokinetic study

Blood samples (5 ml) for measurement of plasma levels of thiotepa and melphalan were taken from an indwelling intravenous cannula placed in an antecubital vein at 0 (pre-infusion), 30 min, 1, 2, 3, 4, 5, 6, 8, 14, 24 and 48 hours after the last dose of drugs Samples were collected in Vacutainers (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey) that contained lithium heparin as an anticoagulant Plasma was separated by centrifugation at 4000 rpm for 15 minutes and frozen at −20 °C until assayed for drug levels Plasma levels of thiotepa and melphalan were determined by validated reversed-phase high performance liquid chromatography (HPLC) with ultraviolet or fluorimetric detection, respectively, as previously described [14, 15] Maximum plasma concentrations (Cmax, μg/ml), and terminal half lives (t½, h) were calculated as 0 693/λ_1, where λ_1 (1/h) is the negative slope of the log-linear terminal (b) phase of the plasma concentration-time profile of melphalan and thiotepa The area under the plasma concentration-time curve (AUC, μg/ml × h) was calculated after the last of the three drug doses, by using a combination of the linear and log trapezoidal rules The relationship between drug exposure and days of mucositis, days of febrile neutropenia, time-to-progression (TTP) and overall survival (OS) of patients was evaluated by constructing scatterplots and fitting the data by linear regression algorithm as a function of either plasma Cmax and AUC of melphalan and thiotepa

Results

Patients

Forthy-eight patients with MBC entered into the study. Median age of the population was 48 years (range 28–59) and median ECOG performance status was 0. Twelve patients had MBC 'ab initio' and thirty-five patients were in first relapse. median disease-free interval from initial diagnosis was 24 months (range 0–108). Median number of metastatic sites was 3 (range 1–5), five patients had positive bone marrow cytology Twenty-three patients (48%) were hormone receptor negative and fourteen patients (29%) were hormone receptor positive; the hormone receptor status was not available in eleven patients. Thirty-one patients (64.6%) had received adjuvant chemotherapy that included epirubicin in eleven patients. The median cumulative dose of epirubicin re-

Table 1 Characteristics of patients

Number of patients	48
Age (years)	
Median	48
Range	28–59
Performance status (WHO)	
Median	0
Range	0–1
Free interval from diagnosis (months)	
Median	24
Range	0–108
Hormonal receptor status (*n*, %)	
Negative	23 (47 9)
Positive	14 (29 1)
Unknown	11 (22 9)
Previous adjuvant treatment (*n*, %)	
Chemotherapy	31 (64 6)
With anthracycline	11 (35 5)
Dominant metastatic site (*n*, %)	
Viscera	32 (66 6)
Bone	16 (33 3)
Soft tissue	36 (75 0)
Bone marrow	5 (10 4)
Number of metastatic site (*n*, %)	
1	7 (14 6)
2	12 (25 0)
⩾3	29 (64 4)
Number of induction courses	
Median	6
Range	4–9
Interval before HDC (days)	
Median	35
Range	20–100
Disease status after induction treatment (*n*, %)	
Complete remission	14 (29 2)
Partial remission	34 (70 8)

ceived before HDC was 540 mg/m^2 (range 450–1170). Eighteen patients had received hormonotherapy in the adjuvant setting After HDC 20 patients were treated with hormonotherapy (Table 1).

Response to conventional treatment and mobilization of circulating hemopoietic progenitors

The induction regimen was ET in 25 patients and GET in 23 patients; after 6 courses of induction chemotherapy 14 patients (29.2%) were in complete remission, and 34 (70.8%) in partial remission The median number of leukapheresis procedure for each patient was 2 (range 1–3) and median number of CD34+ cells/kg collected for each apheresis was 6.3 × 10^6 (range 0.52–20.4).

Toxicity

High-dose chemotherapy was administered after a median of 35 days (range 20–70) from the last cycle of the induction phase. Median number of CD34+ cells/kg reinfused was 6.92 × 10^6 (range 1.53–16.6). The median time for hematologic recovery to a neutrophil count more than 0.5 × 10^9/l was 9.5 days (range 4-19), to platelets more than 20 × 10^9/l and 50 × 10^9/l, 10 days (range 9–32) and 12 days (range 9–33) respectively (Table 2). Neutropenic fever occurred in 81% of patients lasting a median of 6.5 days (range 2–17). No infections were documented Major non-hematologic toxicities were acute and delayed vomiting in 25% and 31% of patients respectively; a grade 3 mucositis requiring total parenteral nutrition occurred in 58.3% of patients and lasted for a median of 5 days (range 3–9) (Table 3).

Table 2 Hematologic toxicity and hemopoietic recovery

	Number of days median (range)
Time to ANC > 500/μl	9 5 (8–19)
Time to platelets > 20,000/μl	10 (9–32)
Time to platelets > 50,000/μl	12 (9–33)
ANC < 500/μl	5 (1–19)
Platelets < 20,000/μl	4 (1–31)
Neutropenic fever	6 6 (2–17)
RBC units transfused, median (range)	3 (1–3)
Platelet units transfused, median (range)	2 (1–3)

Table 3 Non-hematologic toxicity (WHO grade)

	Grade 1	Grade 2	Grade 3	Grade 4
Nausea/vomiting	39 5	54 2	6 3	0
Diarrea	21	25	0	0
Mucositis	4	37 5	58 3	0
Cardiotoxicity	10 4	6 2	4 2	0

Values are as percentage of patients

Median L-VEF was 56% (range 50%–70%) before induction chemotherapy, 55% (range 50%–59%) after induction chemotherapy and was reduced to 51% (range 25%–58%) after high-dose chemotherapy. Following high-dose chemotherapy the L-VEF was less than 50% in 14% of the patients but returned to normal values within 12 months after transplant. Five patients (10%) developed grade 1 cardiotoxicity, three patients (6.2 %) grade 2 and two patients (4.1%) developed symptomatic congestive heart failure. These two patients showed a significant decrease in L-VEF immediately after transplant followed by the appearance of clinical symptoms three months later; both patients completely recovered with specific treatment.

There was one treatment-related death; this patient developed a renal failure at day +27 after PBPC reinfusion and died for acute renal failure and massive gastrointestinal bleeding, the autoptic examination failed to show any residual disease.

Drug distribution and pharmacokinetic–pharmacodynamic relationship

The analysis of drug disposition showed that the C_{max} of thiotepa 200 mg/m^2 and melphalan 160 mg/m^2 were similar (8.91 ± 0.67 vs. 8.1 ± 2.06 μg/ml, respectively), while the AUC values were markedly different: 28.24 ± 2.91 vs. 3.36 ± 0.56 μg/ml h for thiotepa and melpha-

Table 4 Pharmacokinetic parameters of melphalan and thiotepa in breast cancer patients

	Melphalan 160 mg/m^2	Thiotepa 200 mg/m^2
C_{max} (μg/ml)	8 1 ± 2 06	8 91 ± 0 67
AUC (μg/ml h)	3 36 ± 0 56	28 24 ± 2 91
t½β (h)	1 49 ± 0 2	4 15 ± 0 61
CL (l)	36 97 ± 8 02	13 23 ± 1 33
Vdss (l)	38 45 ± 5 34	55 9 ± 7 35

lan, respectively. The higher systemic exposure to thiotepa was associated with a longer terminal half-life as compared to melphalan (4.15 ± 0.61 vs. 1.49 ± 0.2 hour, respectively) and markedly lower clearance (13.23 ± 1.33 l/h vs. 36.97 ± 8.02 l/h) (Table 4). Finally, a significant linear relationship was observed when comparing the C_{max} of thiotepa vs. time-to-progression (r^2 = 0.6) or overall survival (r^2 = 0.56) and the AUC of thiotepa vs. overall survival (r^2 = 0.4). However, no significant relationship were observed between pharmacokinetic parameters of thiotepa and melphalan and treatment-induced toxicities

Response to high-dose chemotherapy and survival

After HDC, 15 of the 34 partial responders were converted into complete remission (conversion rate of 44 1%) and this raised the complete remission rate at the end of treatment to 60.4%.

At a median follow-up of 39 months (range 6–60) the progression-free and overall survival rates are 37.5% and 65%, respectively, with a median progression-free and overall survival of 21 months (95% CI: 17–25) and 38 months (95% CI: 24–60), respectively. Median progression-free and overall survival are 17 months (95% CI: 12–22) and 28 months (95% CI: 24–34) respectively for patients transplanted in PR; median progression-free survival is 30 months (95% CI: 20–40) for patients transplanted in CR while median survival has not yet been reached (Figure 1) Median progression-free and overall survival are 20 months (95% CI: 7–34) and 38 months (95% CI· 16–60), respectively, for patients achieving CR after HDC and 17 months (95% CI. 12–23) and 28 months (95% CI: 12–48), respectively, for patients with persistent PR after HDC, these differences are not significant Twenty patients received hormonotherapy after HDC. Median progression-free and overall survival are 18 months (95% CI. 12–24) and 28 months (95% CI: 22–35), respectively, for patients not receiving hormonotherapy; median progression-free is 30 months (95% CI: 17.5–42.6) for patients receiving hormonotherapy while median survival it is not been reached

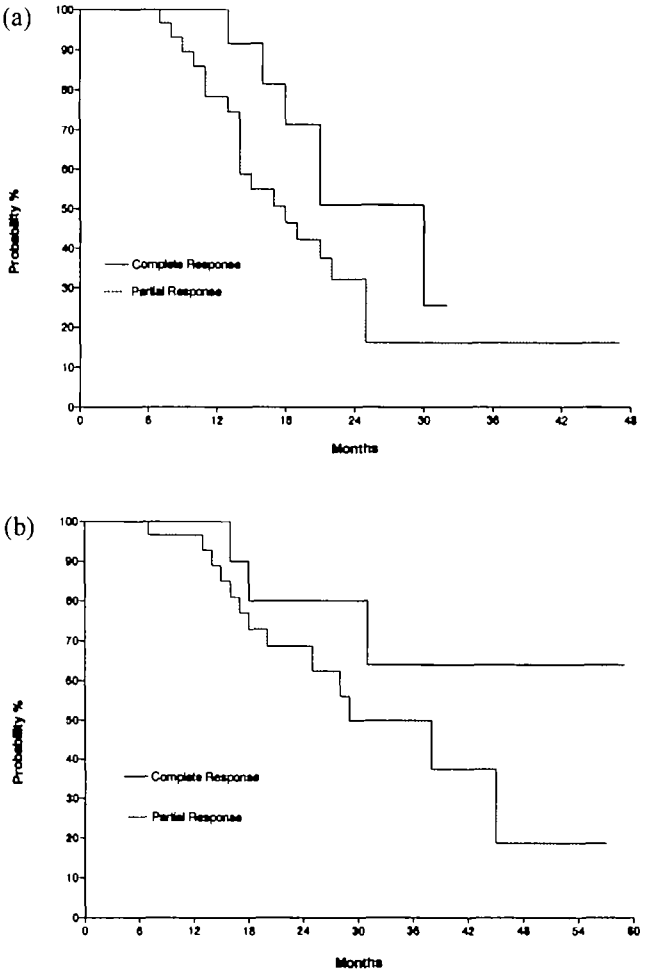

Figure 1 (a) Time to progression, and (b) overall survival according to response to induction chemotherapy

Discussion

High-dose chemotherapy with hemopoietic support as consolidation treatment in chemoresponsive MBC has been extensively explored in phase II and currently in phase III randomized studies [1, 2]. Preliminary results of randomized trials have failed to show a significant benefit for patients receiving HDC, it is therefore clear that high-dose chemotherapy has still to be considered an experimental procedure. Among the possible ways to improve the results we have tested the administration of very active non alkylating-containing regimens in the induction phase followed by double high-dose alkylating agents.

We have already shown that ET and GET are very active, devoid of significant cardiotoxicity [8, 9, 13] and in combination with G-CSF are able to mobilize PBPC [10]. In the present study we have evaluated the toxicity, mainly cardiotoxicity, and the activity of high-dose thiotepa and melphalan as consolidation therapy following induction with ET combinations.

In previous studies of HDC, cardiac complications have been reported in up to 45% of patients and congestive heart failure in 17% of cases [16]. More recently, Hertenstein et al. have reported a 4.7% incidence of cardiac toxicity after bone marrow transplantation with three patients (1.8%) developing severe congestive heart failure [17]. Cardiotoxicity is most commonly associated with the administration of HD cyclophosphamide [18].

Additional risk factors for cardiotoxicity are previous chemotherapy including anthracycline and use of total body irradiation [19]. In our study all patients had received epirubicin-paclitaxel-based treatment. The median cumulative dose of epirubicin received before HDC was 540 mg/m^2 (range 450–1170). Recently we have reviewed our data regarding cardiotoxicity on 105 patients treated with an epirubicin–paclitaxel-containing regimen; the incidence of CHF with this regimens is 2% at cumulative epirubicin doses of 540 mg/m^2 and 7.7% up to 990 mg/m^2 [13]. In the present study the risk of sintomatic cardiotoxicity after consolidation HDC was 4.1% and all the patients fully recovered with appropriate treatment.

Pharmacokinetic profiles of high-dose thiotepa and melphalan were analyzed to investigate possible relationship with toxicity and clinical outcome. The present study demonstrates the lack of interaction of thiotepa on melphalan disposition, since the drug distribution was similar to that previously reported with high-dose melphalan alone [20]. In agreement with published findings, pharmacokinetic parameters of melphalan were not correlated with non-hematologic toxicities [11, 20, 21]. On the contrary, a relationship between thiotepa pharmacokinetics (C_{max} and AUC) and clinical parameters (time-to-progression and overall survival) was observed, suggesting that drug exposure is an important determinant of thiotepa efficacy. This finding underscores the importance of concentration-controlled clinical trials in patients examined with a limited sampling strategy with respect to their plasma levels of thiotepa in order to enhance tumor exposure and increase the likelihood of response In a previous work, the AUC and total body clearance of thiotepa was closely related with neutrophil but not to platelet count [22]. Of note, thiotepa displays a dose-dependent antitumor effect in experimental models *in vivo* [23] and high-dose treatment may represent a realistic strategy in order to overcome the mechanism of resistance of tumor cells, including the saturable glutathione S-transferase (GST)-catalyzed conjugation of thiotepa to glutathione that represents an important factor in the development of drug resistance towards the drug [24]. Finally, after 10 elimination half-lives the serum levels of melphalan are negligible and unable to affect the viability of autologous PBPC; it is therefore appropriate to reinfuse PBPC 18–24 hours after the admistration of melphalan

The achievement of CR before HDC is the most important prognostic factor for progression-free survival [25–27]; therefore the use of very active regimens as induction treatment before HDC is part of the therapeutic strategy. The use of induction regimens not including alkylating agents might reduce the risk of drug resistance to alkylating agents that are the cornerstone of HDC In our study 29.2% of patients were in complete remission after induction treatment with ET or GET and high-dose thiotepa + melphalan was able to induce a conversion rate (from partial to complete remission) of 44 1% thus raising the final CR rate to 60.4%. However, as previously reported by others [25–27], median progression-free survival is more prolonged if the patients are transplanted in CR (30 months vs. 17 months). Comparing the data of TTP and OS of the patients transplanted in CR or achieving CR after HDC or with persistent PR after HDC we observed the better outcome in the patients transplanted in CR status. The achievement of complete remission after HDC did not result in a significant benefit in terms of progression-free and overall survival. The hormonal treatment after HDC does not affect significantly the outcome of treatment. However, patients receiving hormonotherapy show a prolonged TTP (17 months vs. 30 months) and OS ($P = 0.09$).

In conclusion, the present study shows that high-dose double alkylators after induction with epirubicin–paclitaxel combination is feasible, devoid of significant cardiotoxicity and active; moreover pharmacokinetics parameters of high-dose thiotepa might be linked to treatment outcome. Since data from randomized trials have failed to prove superiority of HDC it is therefore important to develop new therapeutic plans with more active induction regimens and HDC combinations with reduced toxicities which allows for multiple dose-dense administrations. Indeed, according with the Norton–Simon hypothesis, a multi-cycle dose–dense chemotherapy may allow to kill first more aggressive cell clones preventing regrowth of the tumor in the intervals between the courses This strategy has been demonstrated feasible without hemopoietic support and encouraging results have been showed in terms of progression-free and overall survival when used as front-line intensive treatment [28] and it has to be compared with HDC as consolidation after conventional treatment.

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