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Original Paper

A Randomised Study Comparing Granulocyte-colony Stimulating Factor (G-CSF) with G-CSF Plus Thymostimulin in the Treatment of Haematological Toxicity in Patients with Advanced Breast Cancer after High Dose Mitoxantrone Therapy

F. Sanchiz and A. Milla

Centre de Radioterapia i Oncologia, Clinica Platón, 21 Platón St, 08006 Barcelona, Spain

54 patients with advanced breast cancer were randomised into a prospective, non-blinded, controlled trial to receive: mitoxantrone 28 mg/m² intravenous day 1 and granulocyte-colony stimulating factor (G-CSF) 5 μg/kg/day subcutaneously days 2 to 16 (n = 27) or the same regimen plus thymostimulin (TS) 50 mg/day intramuscular at days 2 to 16 (n = 27). The median time to reach a neutrophil count greater than 0.5 × 10⁹/l was lower in the G-CSF + TS treated group (9.13 versus 3.24 days; $P < 0.0005$). More patients experienced neutropenic fever in the G-CSF group than in the G-CSF + TS group (59.3% versus 22.2%, $P = 0.0119$). The incidence, duration and severity of clinically or bacteriologically documented infection were lower in patients who received TS. 16 patients (59.3%) in the G-CSF group contracted infection, and 4 patients (14.8%) receiving G-CSF + TS ($P = 0.0016$). These data indicate that the combination of G-CSF and TS is well-tolerated and may enhance haematological recovery following myelosuppressive chemotherapy in patients with advanced breast cancer.

Key words: advanced breast cancer, granulocyte colony-stimulating factor, haematological toxicity, mitoxantrone, myelosuppression, thymostimulin
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INTRODUCTION

MYELOTOXICITY IS a significant complication of standard intensive chemotherapy in the treatment of cancer, often resulting in neutropenia, febrile episodes and life-threatening infection. Following the introduction of recombinant haematopoietic growth factors (rHGF), which stimulate the production of mature myeloid cells and therefore accelerate haematological recovery following myelosuppressive therapy, much progress has been made in this field. With enhanced host defences, chemotherapy regimens may be delivered more effectively so that the patient receives full doses of chemotherapy on schedule. Additionally, improved control of myelotoxicity has allowed the investigation of intensified chemotherapy with the aim of ameliorating response and survival [1].

Granulocyte colony-stimulating factor (G-CSF) is a lineage-specific HGF which is secreted by marrow stromal cells, endothelial cells and fibroblasts. It can be distinguished from other HGFs by its ability to stimulate preferentially the development of neutrophils. G-CSF increases neutrophil count by amplifying production from precursors and by shortening the time taken for mature cells to be released into the circulation from 5 days to 1 day [2]. A recombinant form of human G-CSF expressed in *Escherichia coli* (non-glycosylated recombinant methionyl human G-CSF) has been available for clinical use since 1991. Some clinical trials with recombinant G-CSF have demonstrated that it significantly reduces the incidence, severity and duration of neutropenia induced by myelotoxic chemotherapy. G-CSF, therefore, protects against the risk of infection, reduces the need for hospitalisation and antibiotics, and enables full-dose myelotoxic chemotherapy to be delivered on schedule [3–5]. Recombinant G-CSF is used as an adjunct to intensive chemotherapy regimens where there is a high risk of neutropenic infection.

Thymostimulin (TS) is a bovine thymic extract consisting of a mixture of peptides with a molecular weight of approximately 12 000 daltons. It is an immunomodulatory agent that is capable of stimulating growth and differentiation of T-cells. TS has been shown to induce T-cell proliferation when peripheral blood lymphocytes from elderly subjects [6] and Hodgkin's disease patients [7] are incubated with TS and various plant mitogens specific for T-cells. TS has also been demonstrated to enhance

Correspondence to F. Sanchiz
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T-cell lymphopoiesis significantly, and to reduce the incidence of myelotoxicity in cancer patients treated with polychemotherapy [8–10]. In addition to the above immunomodulatory effects, there is evidence that TS increases T-cell number in human cord blood [11], the proliferation of macrophages from immunodeficient nude mice [12], the cytotoxic activity of NK cells from peripheral blood of normal subjects against K 562 erythroleukaemic cells [13] and reduces the myelosuppression of cyclophosphamide-treated mice [14].

TS and G-CSF have different actions on the haematopoietic system (TS is a non-specific immunomodulatory agent and G-CSF specifically stimulates neutrophil production). Therefore, it can be hypothesised that used in combination an additive or synergistic effect should be observed on haematopoietic recovery.

This study was undertaken to assess whether a combination of TS and G-CSF is more effective than G-CSF alone in enhancing haematological recovery, following myelosuppressive chemotherapy in patients with advanced breast cancer treated with intensive doses of mitoxantrone. This agent is known to have significant myelosuppressive activity resulting in an increased risk of infectious complications [15, 16].

PATIENTS AND METHODS

A prospective, randomised, non-blinded, controlled trial was conducted to determine whether TS reduces chemotherapy-induced toxicity in patients treated with high dose mitoxantrone and G-CSF. From June 1992 to December 1993, 54 patients with advanced breast cancer were entered in the trial.

Eligibility criteria

Patients who met the following inclusion criteria were eligible for entry into the trial: advanced breast cancer with at least one measurable lesion, age under 55 years, performance status between 0 and 2 (WHO scale), adequate bone marrow function (haemoglobin >9 g/dl, platelets $>100 \times 10^9$/l and total leucocytes $>4.0 \times 10^9$/l), normal hepatic and renal function (serum bilirubin <1.2 mg%, serum transaminases < twice normal value, serum creatinine <1.2 mg%, BUN <50 mg%) and informed consent.

Treatment schedule

The 54 advanced breast cancer patients were randomly assigned (by means of tables of random numbers) to one of two treatment groups. In the first group, 27 patients received mitoxantrone (Novantrone®, Lederle) at a dose of 28 mg/m^2 intravenous (i.v.) on day 1 and G-CSF (Filgrastim: Neupogén®, Roche and Granulokine®, P.E.N.S.A.) at a dose of 5 μg/kg/day subcutaneously (s.c.) on days 2 to 16. In the second group, 27 patients received the same regimen plus TS (TP-1® Serono) at a dose of 50 mg/day intramuscularly (i.m.) on days 2 to 16.

If leucocyte counts $>8.0 \times 10^9$/l and absolute neutrophil count $>2.0 \times 10^9$/l were obtained in two consecutive samples before day 16, G-CSF or G-CSF plus TS were withdrawn.

Inclusion and evaluation criteria

Who criteria for assessment of performance status, toxicity and responses were used [16].

The following endpoints were included in the analysis: leucocyte toxicity, which was assessed according to: (1) neutrophil profile, (2) time (in days) to recovery of neutrophil counts greater than 0.5×10^9/l, (3) incidence of neutropenic fever (T^a > 38°C and neutrophils $<0.5 \times 10^9$/l), (4) incidence of infection (documented clinically or bacteriologically); platelet toxicity and number of transfusions; number of erythrocyte transfusions; response rate following chemotherapy.

Statistical analysis

The Fisher's Exact Test and the Chi Square Test with Yates's Correction [17] were used to compare qualitative variables. The Mantel–Haenszel test with Fleiss correction was used to compare qualitative variables in repeated measures [18]. To analyse quantitative repeated measures, ANOVA for repeated measures was used [19]. To calculate and compare the cumulative hazard function, the Mantel–Cox test was performed [20]. Shapiro–Wilk's test was used to assess Gaussian adjustment [21]. Homogenicity of variances was tested using the Levene test [22]. The Mann–Whitney test [23] was used to compare differences in quantitative variables within two groups. All tests were two-tailed. α level was fixed at 0.05.

The statistical analysis were performed using Jeppsen 486/66 computers with the following programmes: BMDP Dynamic v 7.0 (BMDP Statistical Software Inc, Los Angeles, California, U.S.A., 1993), and BMDP New System (BMDP Statistical Software Inc, Los Angeles, California, U.S.A., 1994) for graphic visualisation.

RESULTS

For all patients, results are referred to the first cycle of chemotherapy.

Epidemiological data and baseline characteristics

The treatment groups were comparable with regard to age, metastatic sites, previous treatment and baseline haematological parameters. No statistically significant differences were detected that might indicate a lack of homogeneity between groups (Table 1).

Chemotherapy-induced toxicity

(1) *Haematological toxicity.* The difference in the absolute neutrophil count (ANC) between the two groups was statistically significant ($P = 0.0005$). The neutrophil profile is shown in Figure 1(a). The observed valley in the G-CSF + TS group between days 12 and 16 is due to the withdrawal of patients who reached $>8 \times 10^9$/l leucocytes and $>2 \times 10^9$/l neutrophils in two consecutive samples. Figure 1(b) shows in detail the grade IV neutrophil toxicity profile. There were significantly fewer days of grade IV neutropenia in patients receiving G-CSF + TS (median 2 days) compared with those receiving G-CSF alone (median 10 days) ($P < 0.0005$).

Figure 2 shows the percentage of patients with grade IV neutropenic toxicity throughout the treatment period, indicating benefit in favour of the combined treatment group (overall significance $P = 0.0002$, Mantel–Haenszel test). The probability of overcoming neutrophil grade IV toxicity throughout time is shown in Figure 3, with the G-CSF + TS group having an earlier probability of doing so (median 9.13 versus 3.24 days, $P < 0.0005$).

Lymphocyte counts were similar in each treatment group without statistical significance.

Grade IV thrombocytopenia (platelets $<25 \times 10^9$/l) did not occur in any patient receiving G-CSF + TS, and only in 7.4% of patients receiving G-CSF alone (P = NS). No patient in the G-CSF +TS group required platelet transfusions compared with 2 in the G-CSF alone group (P = NS).

Transfusions of erythrocytes were required by more patients receiving G-CSF alone than G-CSF + TS [8 (29.6%) versus 1 (3.7%)]. The difference was significant ($P = 0.02$).

Table 1. Characteristics of patients

	No. of patients (%)	
	G-CSF	G-CSF + TS
Total entered	27	27
Age (median, years) (range)	46 (32–54)	46 (38–54)
Metastatic sites		
Liver	6 (22%)	9 (33%)
Lung	12 (44%)	9 (33%)
Bone	7 (26%)	5 (19%)
Soft tissues	2 (7%)	2 (7%)
Loco-regional	0	1 (4%)
Lung–liver	0	1 (4%)
Prior radiotherapy	11 (41%)	13 (48%)
Prior hormonotherapy	13 (48%)	8 (30%)
Prior chemotherapy	26 (96%)	25 (93%)
Adjuvant only	17 (63%)	13 (48%)
Metastatic only	3 (11%)	3 (11%)
Adjuvant + metastatic	6 (22%)	9 (33%)
Prior therapy (overall)		
Antracyclines	9 (33%)	4 (15%)
No antracyclines	17 (63%)	21 (78%)
Hormonal only	1 (4%)	2 (7%)
Haematological parameters (mean, baseline) (range):		
Leucocytes (×10⁹/l)	8.107 (6.3–9.1)	8.078 (6.1–9.1)
Neutrophils (×10⁹/l)	5.213 (3.44–6.468)	5.357 (4.0–7.642)
Lymphocytes (×10⁹/l)	2.894 (1.44–4.59)	2.984 (1.278–8.918)
Platelets (×10⁹/l)	156.3 (110–212)	150.4 (114–213)
Haematocrit (%)	40.4 (37–44)	40.1 (37–44)

(P = NS).

(2) *Neutropenic fever.* A significantly lower incidence of chemotherapy-induced neutropenic fever was observed in patients who received TS in addition to G-CSF than in those who received G-CSF alone (G-CSF: 16 [59.3%] versus G-CSF + TS: 6 [22.2%]; P = 0.0119).

(3) *Infection.* The incidence, duration and severity of infection (documented clinically or bacteriologically) were significantly lower in the G-CSF + TS treatment group. Of the 27 patients receiving G-CSF, 16 (59.3%) contracted infection compared with only 4 patients (14.8%) in the G-CSF + TS treatment group (P = 0.0016). The duration of infection was measured according to the number of days required to cure the infection (48 h without fever and negative cultures). A median of 10 days was reported in the G-CSF group compared with 5.5 days in the G-CSF + TS group (P = 0.0162). Figure 4 shows the WHO grades of infection observed in the two treatment groups. Only 1 patient receiving G-CSF + TS was reported to have grade II infection compared with 11 patients receiving G-CSF alone, and no G-CSF + TS-treated patients had grade III infection, which occurred in 3 patients treated with G-CSF alone. The documented sites of infection were similar in each group (P = NS) (Table 2).

G-CSF and TS were well tolerated without adverse events related to these drugs.

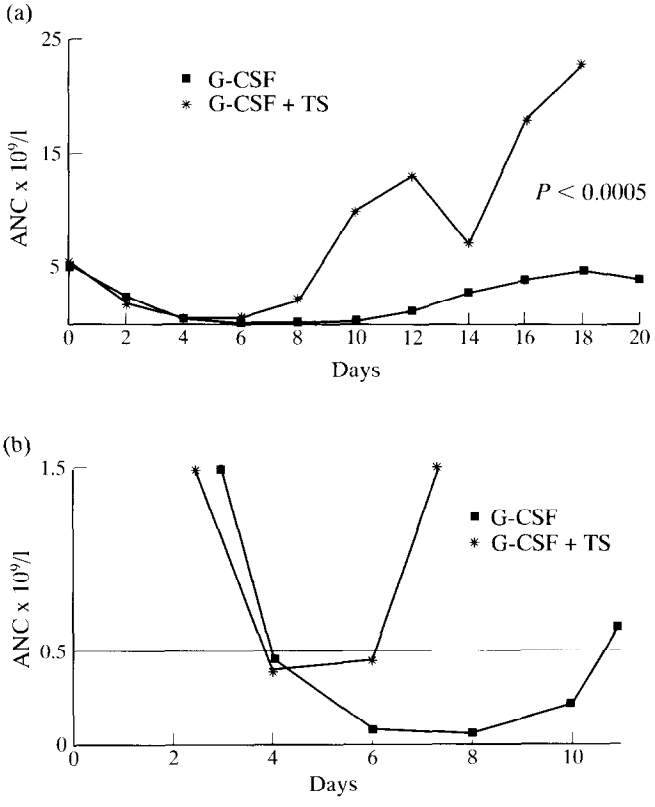

Figure 1. (a) Neutrophil profile (ANC, absolute neutrophil count). (b) Detail of the grade IV toxicity ANC profile of (a).

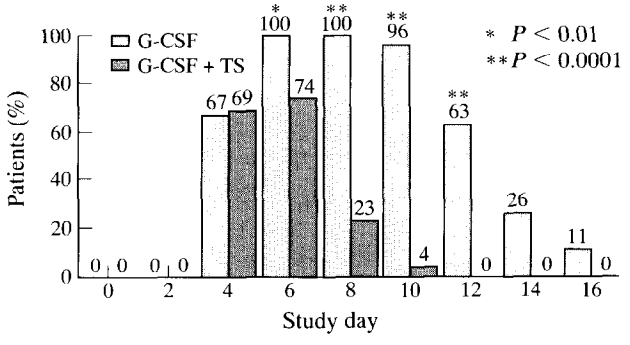

Figure 2. Percentage of patients with grade IV neutropenic toxicity.

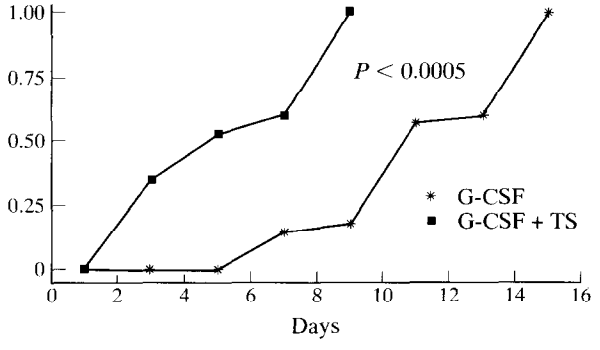

Figure 3. Cumulative hazard functions of overcoming neutrophil grade IV toxicity (WHO).

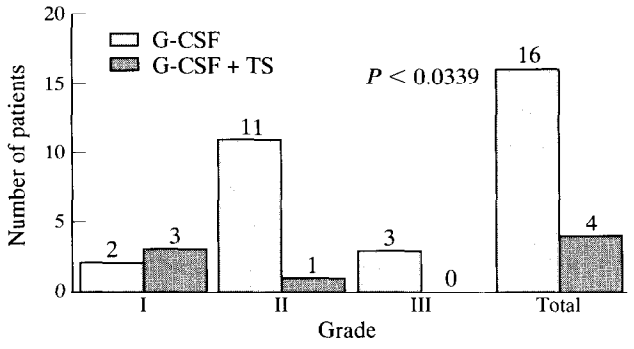

Figure 4. Grade of infection.

Table 2. Incidence of different infection types by treatment group

	G-CSF (n = 16)	G-CSF + TS (n = 4)
Urinary	7 (43.8%)	3 (75%)
Pulmonary	7 (43.8%)	1 (25%)
Mucocutaneous	2 (12.5%)	0

(P = NS)

Response rate to chemotherapy

Responses to mitoxantrone chemotherapy are shown in Table 3. There was no statistical significance difference between groups.

DISCUSSION

This study shows that in patients with advanced breast cancer treated with high doses of mitoxantrone, a combination of TS and G-CSF was more effective than G-CSF alone in reducing the incidence of chemotherapy-induced haematological toxicity, neutropenic fever and infections.

TS has previoulsy been shown to have a stimulatory effect on T-cell lymphopoiesis, and the activity was sufficiently potent to overcome chemotherapy-induced immunosuppression in patients with incurable gastrointestinal cancer treated with 5-fluorouracil, vincristine and methyl-CCNU [8]. TS has also been demonstrated to significantly reduce the incidence of myelotoxicity in breast cancer patients as well as episodes of infection compared with controls [9]. In a further study conducted in patients with small cell lung cancer [10], TS was demonstrated to reduce myelosuppression, fever and documented infectious episodes significantly. This allowed drugs to be given in higher doses and at shorter intervals which appeared to result in a significant improvement in survival and complete response rate. Pavesi [24] have recently demonstrated that TS significantly reduces chemotherapy-induced haematological toxicity in patients with metastatic breast cancer. The enhancement of immune reactivity induced by TS has also been associated with a significantly decreased incidence of postoperative sepsis and mortality, resulting in a reduction in antibiotic requirements and hospitalisation [25, 26].

Table 3. Response to chemotherapy in G-CSF and G-CSF + TS treatment groups

	G-CSF	G-CSF + TS
CR	0	0
PR	0	1
SD	4	6
NR	23	20

CR, complete response; PR, partial response; SD, stable disease; NR, no response (P = NS).

G-CSF has been demonstrated to reduce the risk of infection associated with neutropenia [4]. In a randomised controlled phase III trial [27], G-CSF increased neutrophil counts 16-fold compared with controls, and reduced the incidence of fever, infection, antibiotic requirements and hospitalisation. G-CSF use has also been associated with an acceleration in neutrophil count recovery and reductions in the duration of i.v. antibiotic use, febrile days and hospitalisation after high-dose chemotherapy and autologous bone marrow transplantation [28]. More recently, it has been demonstrated that the use of G-CSF-primed peripheral blood progenitor cells (PBPC) results in a significant reduction in the duration of neutropenia, in antibiotic use and hospital resources [29].

The improved effect of the TS + G-CSF combination may result from complementary actions for the two drugs on the haematopoietic system. Whereas TS has a non-specific immunomodulatory effect involving T-cells, macrophages, NK cells and secondary cytokine secretion [6, 7, 11–13], G-CSF preferentially stimulates neutrophil growth and development [30]. When administered in combination, an additive effect is observed, which results in a reduction in the time to reach more than 0.5×10^9/l neutrophils, in the episodes of neutropenic fever and in the incidence, duration and severity of clinically or bacteriologically documented infection. The use of TS in combination with G-CSF was well tolerated and was not associated with any adverse effects in this study. The clinical applications of TS + G-CSF may extend beyond stimulating haematopoiesis due to the immunomodulatory effects of these drugs, thus providing additional opportunities for investigating new combination approaches.

Clinical responses after the first cycle of chemotherapy werepoor (Table 3). Patients were scheduled to receive three cycles of high-dose mitoxantrone if no progressive disease was observed. The results of this chemotherapeutic regimen are currently being analysed.

The reduction in chemotherapy-induced haematological toxicity observed in the present study may enable chemotherapy to be given at higher doses and with shortened cycle intervals, resulting in fewer treatment delays and, possibly, increased survival [1]. Reductions in episodes of neutropenic fever and infections improve quality of life and might also result in less time in hospital and fewer antibiotics. This may have favourable cost implications. These encouraging preliminary results need further investigation.

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