British Journal of Haematology, 1995, **89**, 191–195

SHORT REPORT

Human recombinant granulocyte-macrophage colony stimulating factor (hrGM-CSF) improves double hemibody irradiation (DHBI) tolerance in patients with stage III multiple myeloma: a pilot study

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Received 23 May 1994; accepted for publication 2 September 1994

Summary. Double hemibody irradiation (DHBI) is an alternative treatment of stage III multiple myeloma (MM) in patients aged over 55 years. Toxic side-effects such as myelosuppression are a severe limiting factor to its use. We performed DHBI associated with human recombinant granulocyte-macrophage colony stimulating factor (hrGM-CSF) as support therapy in 10 patients with stage III MM to improve the tolerance to this treatment.

Ten patients received subcutaneously 5 μg/kg/d of hrGM-CSF during 2 weeks after each course of hemibody irradiation. All these patients had stage III MM: eight previously received chemotherapy, six of them were regarded as patients with refractory MM and two with relapse. Two patients received DHBI as first-line treatment.

hrGM-CSF increased safety and tolerance of DHBI. GM-CSF support reduced the mean time between upper body irradiation (UBI) and lower body irradiation (LBI): 41 *v* 108 d in a cohort of 32 patients previously treated without growth factor support. Overall there was no lethal infection with hrGM-CSF or granulocytopenia ($5{\cdot}0 \times 10^9$/l *v* $0{\cdot}4 \times 10^9$/l at day 15 in patients without growth factor). hrGM-CSF also reduced stomatitis grading and thrombocytopenia (90×10^9/l *v* 45×10^9/l at day 15). Furthermore, hrGM-CSF increased blood colony forming unit-granulocyte macrophage (CFU-GM) and was well tolerated in all but one patient.

hrGM-CSF reduces toxic side-effects of DHBI, thus providing an effective treatment in patients with advanced and resistant MM.

Keywords: multiple myeloma, double hemibody irradiation, human recombinant GM-CSF.

Treatment of patients with advanced multiple myeloma (stage III in the Durie and Salmon staging) is a difficult challenge. Median survival, disease-free survival, response rate and analgesic effects must be considered in therapeutic assessment. Little progress has been observed following the introduction of alkylating agents. In patients with *de novo* MM, multi-institutional controlled trials failed to show a therapeutic advantage of various multidrug regimens when compared with melphalan and prednisone (MP) (Gregory *et al*, 1992). In patients with refractory MM median survival is short with chemotherapy including vincristin, adriamycin and dexamethasone (VAD regimen), or cyclophosphamide and etoposide or high-dose melphalan. Toxicity and mortality caused by severe myelosuppression are the main limiting factors, and the quality of life of these patients is very poor.

Multiple myeloma is radiosensitive: Bergsagel (1971) estimated that 10 Gy would reduce the tumour mass by 3 log. Recent progress has been obtained with autologous or allogeneic bone marrow transplantation using, in most cases, total body irradiation as conditioning regimen. An alternative radiation therapy is double half-body irradiation (DHBI). It has been shown in dogs that a single-dose irradiation of hemibody is followed by a recirculation of stem cells and repopulation of the irradiated bone marrow within 15–20 d (Nothdurft *et al*, 1984, 1989). These observations support the concept that DHBI could be an equivalent of

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autologous bone marrow transplantation without the requirement of peripheral stem cells collection.

DHBI has been proposed for the treatment of solid tumours, lymphomas and MM (Jaffe *et al*, 1979). We previously reported the long-term results of DHBI in 32 patients without hrGM-CSF showing similar results when compared to conventional protocols (Troussard *et al*, 1988; Troussard & Leporrier, 1991). In 19 patients DHBI was the first-line therapy; all these patients had stage III MM and bone pain unrelieved by major analgesics. The overall median survival was 25 months and the analgesic effect obtained had a mean duration of 15 months. In this series we obtained two complete remissions (CR) with a relapse 30 months after DHBI and a persistent CR 15 months after irradiation. However, tolerance of the two consecutive irradiations was poor, with severe pancytopenia in 44% of patients. Four patients died from infection 3 months after DHBI: one septicaemia, one tuberculosis and two pulmonary infections. DHBI also induced severe stomatitis in all cases. We also reported the results in 13 patients with primary resistant or relapsing MM treated with DHBI as second-line treatment: analgesic effect was present in all but one patient with a mean duration of 5 months; the overall median survival was 6 months, comparable to the VAD regimen.

In the present pilot study we treated 10 patients with DHBI, and hrGM-CSF support to reduce toxic side-effects of irradiation.

MATERIALS AND METHODS

Patients. 10 patients underwent DHBI with hrGM-CSF for stage III multiple myeloma according to the clinical staging system of Durie & Salmon (1975).

The diagnosis of MM was established when at least two of the following criteria were present: (1) a paraprotein detectable in serum of urine, (2) >10% plasma cells in bone marrow, and (3) osteolytic and/or osteoporotic bone lesions compatible with MM. Primary resistant or relapsing MM was defined when one or more of the following criteria were fulfilled: (1) at least a 25% increase in the serum M component concentration when compared with the pre-treatment or pre-response value; (2) a 100% increase in 24 h urinary Bence Jones protein excretion when compared with pre-treatment or pre-response value; (3) serum calcium >3 mmol/l, and/or (4) progression of osteolytic lesions.

Eight patients received chemotherapy as first-line treatment: all the patients received MP and two patients received VAD or VMCP-VBAP regimen in cases of primary resistance or relapse. Out of eight patients, six were progressive and two relapsed, with one patient resistant to MP. Chemotherapy was given for a mean of 24 months (13–52) with a mean number of cycles of 12 (8–19). Two patients underwent DHBI as first-line treatment: both had hypercalcaemia and elevated serum $\beta 2$ microglobulin at diagnosis.

All the patients gave their written informed consent.

Double hemibody irradiation (DHBI). As previously described (Troussard *et al*, 1988; Troussard & Leporrier, 1991), total body irradiation, delivered by a Sagittaire 25 MeV linear accelerator, was given in two stages of hemibody irradiation separated by a mean interval of 7 weeks. The border between UBI and LBI was arbitrarily fixed at the umbilicus. The patients were placed in a dorsal decubitus position and a single divided dose was delivered by equal anterior and posterior beams. A dose of 8 Gy was delivered with pulmonary and buccal protection after 6·5 Gy. The starting rate was 50 cGy/min and the mean session time was 15 min. The upper body was treated first because it was usually the more painful. Prior to each session anti-emetics were given to prevent nausea and vomiting. The second irradiation was not given until the granulocyte count reached $1{\cdot}5 \times 10^9$/l and the platelet count reached 100×10^9/l. Blood cell counts were performed weekly until the appropriate values were obtained.

Human recombinant granulocyte-macrophage colony stimulating factor (hrGM-CSF). hrGM-CSF (Schering Plough, France) was delivered at a dose of 5 μg/kg in a daily subcutaneous infusion from day 0 to day 15 after UBI and LBI. Potential side-effects were clinically recorded and graded according to the W.H.O. classification.

Peripheral and bone-marrow granulocyte-macrophage progenitors (CFU-GM) studies. The CFU-GM were cultured using the technique initially described by Pike and Robinson. The results were expressed as the number of colonies per ml of peripheral blood.

Response criteria. Response criteria were those used by the Southwest Oncology Group.

RESULTS

Ten patients, seven males and three females, with a mean age of 63 years (49–71), were entered into this study. All patients had stage IIIA MM at the time of DHBI. The mean time between diagnosis of MM and DHBI was 26·3 months (2–63). The first-treated hemibody was upper body in four and lower body in six patients. Out of 10 patients, nine received the complete treatment. The analysis was updated in January 1994.

Toxic side-effects

We compared the toxic side-effects with a historic cohort of 32 patients treated without hrGM-CSF support.

Haematological toxicity. The pre- and post-DHBI blood counts of patients treated with hrGM-CSF are shown in Fig 1. As expected, neutrophils, eosinophils and monocytes were sustained during the 2 weeks with hrGM-CSF. The changes over time are also shown in Fig 1. For the first hemibody the mean neutrophil count was, respectively, 2·7, 4·5, 7·7 and $2{\cdot}9 \times 10^9$/l before and at days 8, 15 and 21 after irradiation, compared to 2·4, 1·2, 1·0 and $0{\cdot}8 \times 10^9$/l in patients without hrGM-CSF support (Table I). For the second hemibody the mean values were 2·8, 3·0, 5·0 and $1{\cdot}9 \times 10^9$/l. As a consequence, no infection was detected, compared to 11 in the group without GM-CSF. Thrombocytopenia was obvious in all patients: platelet transfusions were required in 7/9 treated patients with a mean number of transfused platelet units of six per patient.

The mean time between UBI and LBI was 41 d (28–50). No irradiation was delayed in patients treated with

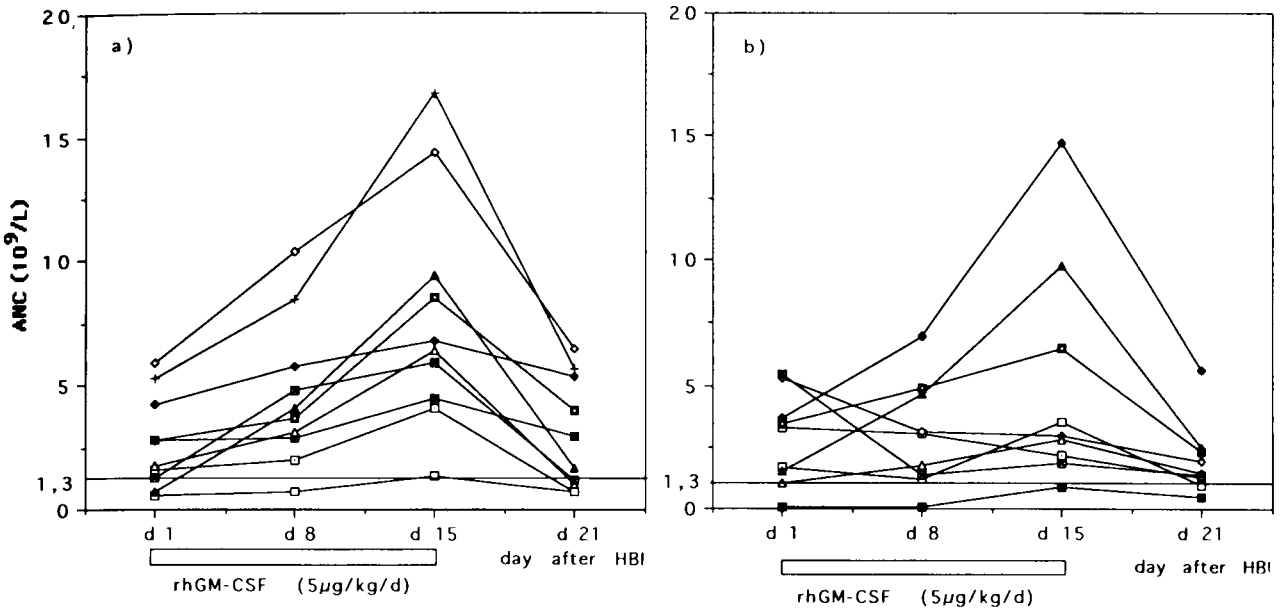

Fig 1. Granulocyte counts after first (a) and second (b) hemibody irradiation (HBI) in patients with rhGM-CSF support.

hrGM-CSF. In contrast, 13 patients received UBI or LBI 10–18 weeks after the first irradiation.

Other toxicities. hrGM-CSF was well tolerated except by three patients who reported a slight exacerbation of bone pain; however, it was difficult to distinguish between bone pain related to MM and/or a toxic side-effect of hrGM-CSF. Eventually, pain decreased in all patients after completion of hrGM-CSF treatment, and major analgesics were stopped in

Table I. DHBI in patients with and without hrGM-CSF support.

hrGM-CSF:	Absent		Present	
No. of patients	32		10	
DHBI as first-line therapy	19		2	
DHBI as second-line therapy	13		8	
Time Dg/DHBI (months)	8	(1–37)	26	(2–63)
Monoclonal Ig (g/l)	43	(16–74)	30	(7–54)
β2 microglobulin (μg/l)	4	(1·2–7·1)	6	(3·2–8·5)
LDH (U/l)	142	(110–310)	255	(154–400)
Median granulocytes ($\times 10^9$/l) after first hemibody irradiation				
Day 0	2·4	(0·6–6·3)	2·7	(0·5–5·9)
Day 8	1·2	(0·0–1·4)	4·5	(0·7–10·4)
Day 15	1·0	(0·0–1·4)	7·7	(1·3–16·7)
Day 21	0·8	(0·2–2·1)	2·9	(0·6–6·4)
Median time (days) between UBI and LBI	108	(28–482)	41	(28–50)
Median granulocytes ($\times 10^9$/l) after second hemibody irradiation				
Day 0	2·0	(0·7–5·9)	2·8	(0·07–5·4)
Day 8	1·1	(0·03–1·6)	3·0	(0·04–6·9)
Day 15	0·4	(0·03–0·4)	5·0	(0·8–14·7)
Day 21	0·5	(0·1–1·6)	1·9	(0·5–5·6)
Treatment achieved	25		9	

all cases. Exacerbation of a previously diagnosed supraventricular arrythmia was observed in one patient after 13 d – hrGM-CSF treatment was discontinued and the second hemibody irradiation was not performed. Stomatitis was observed in all cases but was of only grade I with hrGM-CSF as compared to grade 4 in patients without growth factor. In addition, hrGM-CSF had no influence on non-haemopoietic DHBI-related toxicity (nausea, vomiting and alopecia).

Follow-up

The overall follow-up was 11 months (2–33): 7·5 months (5–13) in patients with a previous chemotherapy treatment and 24 months (18 and 30) when DHBI was used as first-line treatment. 5/10 patients died from refractory MM at 2, 5, 6, 6 and 11 months after the completion of DHBI. Interestingly, these patients had reduced bone pain at the time of death. Five patients are alive at 35, 22, 17, 8 and 5 months after treatment.

Tumour response

In two patients with *de novo* multiple myeloma we observed one RC, with a relapse at 33 months treated by VAD regimen, and one very good response persistent at 22 months. In the eight patients receiving DHBI as a second-line treatment we obtained seven partial responses with a decrease of M component from 45% to 85% and one minor response.

Blood CFU-GM before and after DHBI

After the first hemibody irradiation (eight patients tested) mean peripheral blood CFU-GM increased from 12 CFU-GM/ml before irradiation to 35 CFU-GM/ml at day 15 after the first hemibody irradiation. This increase was obvious in three patients, whereas in two additional patients peripheral CFU-GM reached the pre-irradiation values. No beneficial effect was observed in three patients.

After the second hemibody irradiation (six patients tested) the mean peripheral CFU-GM rose from 5 to 17 CFU-GM/ml at day 15. The increase was dramatic in three patients and in two additional patients peripheral CFU-GM reached pre-irradiation values. In contrast, this effect was not observed in three patients who underwent DHBI without hrGM-CSF.

DISCUSSION

DHBI is regarded as an alternative treatment for patients with advanced stage III MM (Jaffe *et al*, 1979). In a prospective and randomized trial DHBI was ineffective as consolidation treatment in patients who achieved remission after VMCP/VBAP chemotherapy (Salmon *et al*, 1990). In contrast, we showed in 18 patients treated with DHBI as first-line therapy an overall median survival of 25 months (Troussard & Leporrier, 1991). However, the main limiting factor was haematological toxicity, which was observed in eight patients (44%). In patients non-responsive to chemotherapy, or in relapse, we and others showed that DHBI-related myelotoxicity is clearly higher (Jaffe *et al*, 1979; MacKenzie *et al*, 1992; Singer *et al*, 1989; Rostom *et al*, 1984). In 41 patients with melphalan-resistant MM, pancytopenia occurred in all patients with a nadir within 3 weeks after hemibody irradiation; myelosuppression was more pronounced after the second procedure as reflected by blood product requirements (Singer *et al*, 1989). In this pilot study we showed that hrGM-CSF associated with DHBI reduced the haematological toxicity when compared to 32 patients receiving DHBI without rhGM-CSF support. First, neutrophil granulocytes counts were sustained during the 2 weeks and after the completion of hrGM-CSF treatment. In contrast, in the group without hrGM-CSF granulocytopenia was severe and protracted in all patients, with a mean nadir at day 21 for the first hemibody irradiation and at day 35 for the second hemibody irradiation. Secondly, four severe infections occurred in the 18 first-line (22%) and seven in the 13 second-line (54%) DHBI-treated patients without rhGM-CSF support, whereas we did not record any infection in patients with rhGM-CSF. In addition, we observed a significant reduction of the mean number of transfused platelet units (6 versus 15 in patients without rhGM-CSF support). hrGM-CSF reduced median time between UBI and LBI: 41 d (28–50) in the group with hrGM-CSF compared to 108 d (28–482) in the group without hrGM-CSF. All but one patient achieved complete treatment, compared to only 50% of patients without rhGM-CSF (Jaffe *et al*, 1979; Troussard *et al*, 1988, 1991; MacKenzie *et al*, 1992; Singer *et al*, 1989; Rostom *et al*, 1984). This effect could be of clinical relevance, because achieving complete treatment and decreasing the mean interval-time between UBI and LBI could result in a better objective response and prolonged disease-free survival as well as overall survival in selected patients (Singer *et al*, 1989).

Non-haematological toxic side-effects were dramatically reduced by rhGM-CSF: severe stomatitis was observed in all cases with DHBI but was of grade I in rhGM-CSF-treated patients, compared to grade III–IV in patients without growth factor support. Jaffe *et al* (1979) also noted severe stomatitis, requiring hospitalization in 2/11 patients; they then employed an anterior cavit shield in following patients.

hrGM-CSF acts as a potent growth factor both *in vitro* and *in vivo* assays: it stimulates proliferation and maturation of myeloid progenitor cells, enhancing neutrophilic and eosinophilic granulocyte counts as well as monocyte counts. To our knowledge, hrGM-CSF has never been used after DHBI. Many of the proposed therapeutic uses emphasize the ability of hrGM-CSF to allow higher drug dosage in cancer treatment and bone marrow transplantation. It has also been reported to reduce the duration of neutropenia and the severity of infections. An explanation of the shortening of mean time between UBI and LBI could be that hrGM-CSF increased peripheral CFU-GM at day 15 after the first and second hemibody irradiation. Experimental data in dogs showed that a 11·7 Gy irradiation of the upper hemibody was followed by an increase in the proliferation and differentiation of granulocyte-macrophage progenitor cells (GM-CFC) in the protected bone marrow (Nothdurft *et al*, 1989). Repopulation by the GM-CFC of the irradiated sites from the protected bone marrow became evident at day 7 after UBI and at day 21 after LBI. Within 370 d all the bone marrow irradiated sites had regained their normal GM-CFC

values. In our patients hrGM-CSF clearly increased the mean peripheral CFU-GM at day 15: from 12 to 35 CFU-GM/ml after the first hemibody irradiation and from 5 to 17 CFU-GM/ml after the second hemibody irradiation. Unfortunately we were unable to demonstrate an increase in bone-marrow CFU-GM with either protected or unprotected or first or second hemibody irradiation.

The role of cytokines in the growth of myeloma cells has been investigated previously. Paracrine or autocrine regulation of the growth and differentiation of myeloma cells by IL-6 has been suggested *in vitro*. The effect of hrGM-CSF is debatable: no significant proliferation of plasma cells was noted following hrGM-CSF, G-CSF, M-CSF, IL-1a, IL-1b, IL-2 or IL-4 treatment (Anderson *et al*, 1989). In contrast, significant proliferation was induced by IL-3 or IL-5 (Anderson *et al*, 1989). Another study showed that hrGM-CSF was a strong stimulator of *in vitro* myeloma cell proliferation by potentiating the response of myeloma cells to IL-6 (Portier *et al*, 1993). The clinical relevance of these *in vitro* findings remains to be confirmed, when GM-CSF is employed in multi-centre trials to improve peripheral blood apheresis in autologous bone marrow transplantation. Interestingly, in our study we did not notice any apparent stimulating effect of the hrGM-CSF on bone marrow myeloma cells; nor did we record any M component increase following hrGM-CSF.

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