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Short Communication

Phase II Trial of rhIL-6 (Interleukin-6) Prior to and Concurrently with VAD (Vincristine, Doxorubicin and Dexamethasone) Chemotherapy for Patients with Multiple Myeloma

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We examined the tolerability and safety of interleukin-6 (rhIL-6) when administered prior to and concurrent with vincristine, doxorubicin and dexamethasone (VAD) in patients with progressive multiple myeloma previously treated with VAD alone. Typical rhIL-6-related effects such as fever, chills, acute phase reactions and reversible abnormalities in liver function tests were observed. The study examined whether rhIL-6 predictably modulated indices of myeloma activity. No consistent, predictable change in myeloma-related parameters was documented upon rhIL-6 administration for either 8 days or 11 days prior to and concurrent with VAD. Two patients showed improved sensitivity to VAD chemotherapy when this was administered with rhIL-6. The overall response rate to rhIL-6 and VAD therapy in this study of relapsed and refractory patients was 50%, comparable to our previous experience with VAD alone in this cohort of patients. © **1997 Elsevier Science Ltd. All rights reserved.**

Key words: multiple myeloma, chemotherapy, interleukin-6

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INTRODUCTION

INTERLEUKIN-6 (IL-6) mediates pleiotropic functions via its cell surface receptors, and is a major growth factor in multiple myeloma [1]. *In vitro*, IL-6 stimulates myeloma cell proliferation [2, 3], although debate continues as to whether IL-6 primarily mediates such activity in an autocrine or a paracrine manner. One strategy to try to enhance tumour cell kill by chemotherapy may be to increase the number of tumour cells in a cell cycle during cytotoxic drug administration using tumour stimulatory cytokines. In addition, the priming of tumour cells by cytokines may alter their responsiveness to cytotoxic drugs independently of cell kinetic alterations. Modulation of tumour cell sensitivity to chemotherapy by cytokines has been explored with granulocyte-macrophage colony stimulating factor (GM-CSF) and cytosine arabinoside in acute myeloid leukaemia (AML) [4] and with interferon and 5-fluorouracil in colon carcinoma [5], but modulation of chemotherapy in multiple myeloma using the stimulatory cytokine IL-6 has not been previously explored.

This trial of rhIL-6 in conjunction with chemotherapy aimed to evaluate the safety, efficacy and tolerability of rhIL-6 when administered prior to and concurrent with vincristine, doxorubicin and dexamethasone (VAD) in patients with myeloma. The effects of rhIL-6 have been reported in other groups of patients [6–11], but the effects of exogenous rhIL-6 in patients with multiple myeloma have not been hitherto reported. Haematological, immunological and biochemical effects of rhIL-6 in myeloma patients were evaluated.

The combination of VAD as chemotherapy for multiple myeloma was first described by Barlogie and associates in 1984 [12] as a salvage therapy for patients with myeloma refractory to alkylating agents. A modified form of the original regimen has been used at the Christie Hospital,

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Manchester, U.K., and the results have been previously published [13]. In this study, VAD was used in conjunction with rhIL-6 in a cohort of 12 patients with myeloma who had relapsed after previous VAD, or who had disease which was refractory to VAD.

PATIENTS AND METHODS

12 patients aged between 56 and 72 years, with histologically proven multiple myeloma, who had received prior VAD chemotherapy, were enrolled in the study following written, informed consent. Patients were required to have a Karnofsky performance score of ≥60 and creatinine clearance of >35 ml/min. Patients were ineligible if they had evidence of a paraspinal mass or neurological problems related to myeloma. The trial was approved by the local ethics committee.

The initial 6 patients received daily subcutaneous rhIL-6 (Sandoz SDZ ILS 969) for a total of 8 days beginning 4 days prior to commencement of VAD and continuing during the 4 days of chemotherapy. Subsequent patients received an extended schedule of rhIL-6 over a total of 11 days, commencing 7 days prior to VAD and continuing during the 4 days of chemotherapy. Three initial patients received rhIL-6 at 2.5 μg/kg/day; because this dose was very well tolerated, the remaining 9 patients all received 5.0 μg/kg/day.

VAD chemotherapy was administered as a 4-day continuous infusion of vincristine (1.6 mg) and doxorubicin (36 mg/m^2), via a central venous catheter, together with oral dexamethasone (40 mg daily for 4 days). Prophylactic medication with cimetidine, ketoconazole and co-trimoxazole was used for the first 7 days of each cycle. Oral allopurinol was given to patients with renal impairment or high tumour burden.

Following 3 cycles of rhIL-6 + VAD, patients continued with 3 courses of VAD alone unless they had a complete response (CR). Patients whose disease progressed during VAD alone were offered 3 additional cycles of therapy of rhIL-6 + VAD.

Myeloma staging was performed according to Durie and Salmon staging [14]. Baseline investigations included skeletal survey, bone marrow aspirate and trephine, quantitation of immunoglobulins and paraprotein, β2 microglobulin, creatinine clearance, and 24 h urinary light chain excretion. Routine haematology and biochemistry assessments were made during each rhIL-6 + VAD cycle along with serial immunoglobulin, paraprotein and urinary light chain estimations. During the first cycle, bone marrow samples were obtained at baseline (before therapy), at day 0 (following rhIL-6 "priming") and at day +4 (post-completion of rhIL-6 and chemotherapy). Thymidine labelling studies were performed on bone marrow aspirates, but were non-informative. Toxicity was graded according to WHO criteria. Assessment of response was made according to the criteria defined by the Chronic Leukaemia–Myeloma Task Force [15], except that the definition of complete response was that described by Gore and associates [16].

RESULTS

The characteristics of the patients are shown in Table 1. All patients had received prior VAD chemotherapy and many had received prior melphalan and prednisolone. 11 of the patients were in relapse following previous VAD therapy, and 1 patient had primary VAD-resistant disease. Since all patients had been previously treated with VAD chemotherapy, they were usually able to relate symptoms or side-effects to either rhIL-6 administration, or to the chemotherapy.

rhIL-6-related toxicity

The most common rhIL-6-related toxicity consisted of WHO grade 1 or 2 fever ± chills for which paracetamol was generally given with some symptomatic benefit. Mild grade 1 erythema at the injection site was also almost universal. Fatigue was a prominent symptom in all patients, but appeared to be multifactorial with contributions from disease and VAD chemotherapy in addition to rhIL-6 effects. WHO grade 1 headache was noted by 4 patients during rhIL-6 therapy and 1 patient had grade 2 somnolence associated with fever on the first day of rhIL-6 administration, although no subsequent episodes occurred. One patient developed an acute mononeuropathy of the right ulnar nerve of unknown aetiology, which subsequently improved during continued therapy with rhIL-6 and VAD. Two patients experienced marked increases in their bone pain during rhIL-6 administration.

All patients showed a fall in haemoglobin during rhIL-6 + VAD cycles, and 7 patients required transfusion because

Table 1. Patient characteristics

Number	Gender	Age (years)	Myeloma	Prior therapies	Interval between VAD and IL-6 + VAD (months)
1	M	58	IgAκ	VAD, VBAP, MP, XRT	29
2	F	72	IgGκ	VAD, MP, XRT	9
3	M	56	IgGκ	XRT, VAD, C, αIFN, MP	14
4	F	69	IgGκ	MP, VAD	14
5	M	63	IgGλ	MP, VAD, VBAP	12
6	F	54	IgAκ	VAD, MP	50
7	M	63	IgGλ	VAD, MP	82
8	M	56	IgAκ	VAD, C, αIFN, MP, αIFN	12
9	M	51	IgAκ	VAD, MP, αIFN	47
10	M	62	IgAκ	VAD, MP	5
11	M	57	IgGλ	VAD	1
12	M	68	IgGλ	VAD, MP	37

VAD, vincristine, doxorubicin, dexamethasone; MP, oral melphalan and prednisolone; IFN, α-interferon; C, cyclophosphamide; VBAP, vincristine; BCNU, doxorubicin, prednisolone; XRT, regional radiotherapy

of symptomatic anaemia. The aetiology of this is difficult to determine because of contributions from the cytotoxic chemotherapy and the nature of the underlying disease in addition to the likely direct involvement of rhIL-6 [17, 18]. Leucocyte counts, differentials and platelets were not altered by rhIL-6 administration during days prior to VAD. rhIL-6 was implicated in reversible increases in alkaline phosphatase and/or GGT (gamma glutamyltransferase) by at least 1 WHO grade in 8 of the 12 patients. There was no hypercalcaemia induced by treatment with rhIL-6.

Chemotherapy-related toxicity

VAD-related toxicity such as alopecia, mucositis, paraesthesiae, constipation and steroidal effects (e.g. sleeplessness, indigestion) occurred as anticipated [13], and these toxicities were not altered during cycles which were accompanied by rhIL-6. 17 episodes of infection requiring antibiotic therapy occurred in 10 of the 12 patients during the 47 cycles of rhIL-6 + VAD. During 29 cycles of VAD alone, 3 episodes of infection occurred in 10 patients. Since cycles of therapy with VAD alone were not routinely monitored by weekly blood counts, comparative myelosuppressive effects of VAD chemotherapy with and without rhIL-6 could not be evaluated.

Marrow assessment

During the first cycle of rhIL-6 + VAD, bone marrow trephines and aspirates were obtained on day 4 and aspirates were obtained on day 0 and day +4 to try to assess proliferative responses of myeloma plasma cells to rhIL-6. There was substantial variability between patients in terms of plasma cell levels in aspirates (and trephines) at the start of the treatment (Table 2). No consistent pattern in plasma cell levels emerged. In 2 of the patients, there appeared to be a significant increase in the proportion of plasma cells under the influence of rhIL-6 alone although sampling variability may account for these observations.

Response

Whilst this trial primarily aimed to assess feasibility and safety of IL-6 in multiple myeloma when administered prior to and concurrently with chemotherapy, the responsiveness of patients to therapy was also assessed. The time to response to VAD was more rapid (median 6 weeks) [13] than to conventional alkylator therapy (e.g. melphalan and prednisolone), and an initial assessment of response was made following completion of the 3 cycles of IL-6 + VAD. One patient progressed during rhIL-6 + VAD therapy, and 5 other patients (including the single patient with VAD-refractory disease) had stable disease. 5 patients demonstrated a partial response to rhIL-6 + VAD therapy, and one patient showed signs of a response, but did not achieve a formal PR.

The patient with progressive disease and the patient who had shown resistance to both VAD alone and to rhIL-6 + VAD were withdrawn from the study following completion of the initial 3 cycles of rhIL-6 + VAD. The remaining 10 patients received 3 cycles of VAD chemotherapy alone, and the patient who had shown signs of a response after 3 cycles achieved a partial response during this period giving an overall response rate of 50% for the 6 cycles of treatment.

Table 2. Bone marrow assessments

Patient	Day −7	Day −4	Day 0	Day + 4	End of IL-6 + VAD
1		hypo	normo	normo	hypo
		3%	80%	82%	2%
2		hypo	ND	ND	hypo
		8%			9%
3		hypo	hypo	hypo	hypo
		4%	3%	3%	<1%
4		normo	normo	normo	ND
		14%	13%	10%	
5		dilute	normo	normo	normo
		8%	5%	5%	3%
6		normo	normo	normo	hypo
		72%	79%	32%	5%
7	dilute		normo	normo	normo
	5%		38%	21%	4%
8	normo		hypo	hypo	hypo
	85%		92%	92%	>90%*
9	hypo		hypo	hypo	normo
	49%		44%	50%	44%
10	dilute		dilute	hypo	normo
	61%		70%	60%	38%
11	normo		normo	normo	normo
	13%		14%	8%	41%
12	normo		normo	normo	normo
	8%		8%	5%	3%

*Patchy, very heavy infiltration with plasma cells.

Normo, normocellular; Hypo, hypocellular; ND, not done.

Patients 1–6 received rhIL-6 on days −4 to day +3. Patients 7–12 received more prolonged rhIL-6 administration from day −7 to day +3. Patients 1, 2 and 3 received 2.5 $\mu g/m^2$/day, the remaining patients received 5.0 $\mu g/m^2$/day. End of therapy assessment was performed 3 weeks following completion of 3 cycles of rhIL-6 + VAD therapy

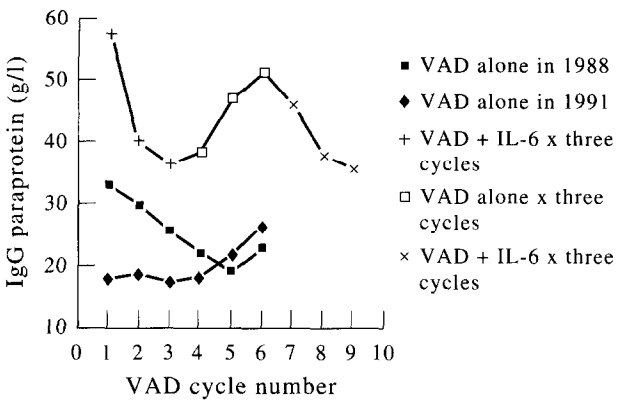

Figure 1. IgG paraprotein changes to VAD ± IL-6 in patient number 5

Having responded to rhIL-6 + VAD, 1 patient developed symptomatic and biochemically progressive disease during the cycles of VAD alone, but showed a further response upon re-introduction of the combination of rhIL-6 and VAD (Figure 1). A second patient who achieved a partial response on rhIL-6 + VAD therapy, had stable disease on VAD alone and rapidly relapsed within 10 weeks of completion. The re-introduction of 3 further cycles of rhIL-6 + VAD produced rapid symptomatic improvement and a 52% fall in paraprotein.

One of the principal concerns at the instigation of this trial was the possibility that the administration of rhIL-6 might accelerate the underlying disease or make it refractory to therapy. Only 1 patient had progressive disease during rhIL-6 + VAD therapy and there was no acute rise in β2 microglobulin, immunoglobulin or calcium associated with rhIL-6 administration. Bone marrow investigations did not identify a pattern of acute changes in myeloma cells in patients, although marked increases in the proportion of myeloma cells were noted in the marrow aspirates of 2 patients. Whilst 2 patients noted worsening in their bone pain during rhIL-6 administration, this occurred in the absence of any other evidence of a disease-specific effect of rhIL-6.

DISCUSSION

This clinical trial examined the tolerability and safety of rhIL-6 when administered prior to and concurrent with VAD chemotherapy in patients with progressive multiple myeloma previously treated with VAD alone. The regimen of rhIL-6 + VAD was generally well tolerated with patients being managed on an out-patient basis. The most prominent toxicities associated with the administration of rhIL-6 were symptoms and signs of an acute phase reaction with fever and malaise.

IL-6 is a growth factor for multiple myeloma cells *in vitro*, and the study also examined whether rhIL-6 predictably modulated clinical indices of myeloma activity such as pain scores, as well as laboratory parameters (serum calcium, β2 microglobulin and immunoglobulin, urinary light chains, bone marrow). No consistent change was documented in any of these laboratory parameters of myeloma activity upon rhIL-6 administration for either 8 days or 11 days prior to and concurrent with VAD, although changes in bone pain and bone marrow myeloma cells were seen in 4 individuals. Although there was no clear-cut effect on myeloma-related indices, almost universally rhIL-6 effects were seen on other measured parameters (fever/chills/acute phase reactions, liver function test abnormalities). This development of acute phase responses suggested that significant increases in systemic IL-6 levels were achieved.

Approximately one-third of patients with multiple myeloma have measurable serum IL-6 levels by bioassay or immunoassay [19, 20], and levels of around 5 pg/ml using sensitive immunoreactive techniques are typical [22]. A subcutaneous dose of 5.0 μg/kg of rhIL-6 produces peak levels of around 300 pg/ml and IL-6 levels persist above background levels for approximately 18 h (data not shown). In spite of this, a definable responsiveness of myeloma cells in humans to exogenously administered rhIL-6 has not been identified in this study. It is probable that a measurable effect on myeloma parameters lags significantly behind the acute phase response. The most likely site to observe early rhIL-6-induced myeloma effects is the bone marrow, but unfortunately, *in vivo* assessment is problematic because of the inherent sample-to-sample variability—myeloma by its nature often exhibits variable and patchy marrow involvement. No secondary myeloma-related parameters (immunoglobulins, light chain excretion, β2 microglobulin, calcium) were predictably altered by the 'priming' phase of rhIL-6 administration.

Multiple possibilities for these findings can be cited. The schedule or dose of rhIL-6 employed in the studies here may have been suboptimal for eliciting an *in vivo* definable response from myeloma cells. In particular, the relatively short 'priming' phase of 4–7 days prior to VAD may be too short to allow identifiable disease-related changes to occur. Furthermore, it has been reported that the addition of IL-6 or anti-IL-6 antibodies to myeloma cells *in vitro* does not modulate their Ig production [21, 22]. Such observations may be relevant in explaining the lack of consistent effect of rhIL-6 prior to VAD on serum paraprotein levels. IL-6 is a highly pleiotropic cytokine and its administration *in vivo* triggers a cascade of secondary cytokines whose effects may mask any direct IL-6-mediated change. Finally, myeloma cells are responsive to multiple extracellular signals *in vivo* and development of a response to IL-6 *in vivo* may require regulated supplementary signals.

In 2 patients, rhIL-6 appeared to modulate the sensitivity of the disease to VAD chemotherapy, although the reason underlying these instances have not been identified. Reassuringly, the response rate to VAD chemotherapy did not appear to be notably diminished by rhIL-6 therapy, the response rate of 50% in this small group of previously treated patients being comparable to the 61% response rate to VAD alone seen in a relatively similar group at our centre [13]. Although modulation of tumour cell sensitivity with cytokines such as IL-6 has rationale from *in vitro* work, it is evident that further work is required to identify whether this forms a useful clinical strategy in multiple myeloma.

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