Eur J Cancer, Vol. 29A, No. 12, pp. 1700–1707, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00
Pergamon Press Ltd

Recombinant Human Interleukin-4 (rhu IL-4) Administered by the Intravenous and Subcutaneous Routes in Patients with Advanced Cancer—A Phase I Toxicity Study and Pharmacokinetic Analysis

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19 patients with advanced cancer were entered into a phase I study of recombinant human interleukin-4 (rhu IL-4). The predominant clinical side-effects included flu-like symptoms, gastrointestinal upset, lethargy and transient hypotension. In addition, there were several cases of capillary leak syndrome. 2 cases of gastrointestinal haemorrhage occurred; this was life threatening in 1 patient. The maximum tolerated dose (MTD) was 400 μg/m²/day. Biochemical toxicity was limited to asymptomatic elevation of liver enzymes suggesting IL-4 induced liver damage. Pharmacokinetic analysis following the intravenous bolus injection has shown that IL-4 is rapidly cleared (mean $T_{1/2}$ = 19 ± 8.7 min) from a small compartment (mean V_d = 4.9 ± 3.68 l) probably indicating that IL-4 is retained in the systemic circulation or at most the extracellular fluid volume. 2 patients with non-Hodgkin lymphomas (NHL) showed a transient response to IL-4 whilst a third patient with NHL showed transient disease progression.

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INTRODUCTION

INTERLEUKIN (IL)-4 IS A 20 kD cytokine produced by activated T cells. Originally described as B cell stimulatory factor by virtue of its ability to co-stimulate B lymphocyte proliferation [1], IL-4 has subsequently been shown to have an extensive range of inhibitory and stimulatory effects on both B and T lymphocytes and appears to play an important role in the modulation of their growth and differentiation. Effects on B lymphocytes include co-stimulation of proliferation with CD 40 monoclonal antibodies [2], induction of surface adhesion molecules [3] and induction of IgM and IgG secretion [4] but a decrease in antigen-specific Ig secretion [5]. Effects on T lymphocytes include co-proliferation in conjunction with PHA and Con A [6] and induction of surface CD8 expression by CD 4+T cell clones [7] but inhibition of IL-2-induced generation of lymphokine-activated killer (LAK) cells [8]. While it would appear that IL-4 regulates many aspects of antigen-specific immunity, the production of IgE by B lymphocytes is the only lymphocyte-mediated function which has clearly been shown to depend on the production of IL-4 *in vivo* [8].

IL-4 receptors are expressed widely [9] and, in addition to its effects on lymphocytes, IL-4 has pleiotropic effects on a wide variety of other peripheral haemopoietic cells, haemopoietic progenitors, endothelial cells and malignant cell lines. These include the induction of class II major histocompatibility complex (MHC) expression on monocytes [10] but a decrease in their cytotoxic activity [11], increased T cell adhesion to endothelial cells [12] and enhanced growth factor supported colony forming unit granulocyte–macrophage (CFU-GM) and burst forming unit erythrocyte (BFU-E) but a decrease in IL-3 supported CFU-GM [13].

Preclinical evidence for an indirect cancer effect of IL-4 is evident from both murine models [14–16] and *in vitro* human studies [17–21]. One experimental approach involved initially transfecting tumour cell lines of various histological types with the IL-4 gene to produce IL-4 and then introducing these cells, alone or mixed with non-transfected tumour cells, into recipient syngeneic and athymic mice [14]. A potent *in vivo* antitumour effect was observed against both the transfected and non-transfected cell lines and the effect was reversed by anti-IL-4 antibody. The antitumour effect seemed to be mediated by an inflammatory infiltrate composed of eosinophils and macrophages and did not appear to be significantly dependent on cytotoxic T lymphocytes or lymphocyte activated killer cells. *In vitro* human studies have shown that IL-4 can promote the proliferation of tumour infiltrating lymphocytes cytotoxic for human autologous melanoma [18]. It has also been shown to inhibit the *in vitro* IL-2-induced proliferation of human B lymphoid malignancies [19–21]. In addition, a phase I clinical study has reported clinical regression in 1 patient with low grade non-Hodgkin lymphoma (NHL) and 1 patient with chronic lymphocytic leukaemia [22]. This encouraging evidence for an antitumour effect by IL-4 has to be weighed against some evidence of effects which could potentiate tumour growth activity [6, 11, 23, 24], including the inhibition of *in vitro* monocyte tumoricidal activity [11] and the enhancement of hairy cell leukaemia DNA synthesis [23].

Administration of IL-4 to non-human primates (data not shown) results in anorexia, weight loss and diarrhoea. Treat-

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Revised 22 Feb. 1993; accepted 1 Apr. 1993.

ment-related haematological findings include a reduction in platelet count and red cell mass, prolonged prothrombin and partial thromboplastin times and onset of disseminated intravascular coagulation. Biochemical findings include decreased total serum protein, albumin, globulins and cholesterol, and increases in lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triglycerides. Subchronic subcutaneous injection of rhu IL-4 to cynomolgous monkeys resulted in a variety of histomorphological changes which included vascular lesions, granulocytic hyperplasia, seminiferous tubular atrophy, splenic lymphoid depletion and thymic atrophy [25]. The vascular lesions, often associated with an infiltration of eosinophils and an intense medial smooth muscle proliferation, were observed in the aorta, brain, caecum, duodenum, gall bladder, heart, ileum, kidney(s), liver, lung, mesenteric lymph node(s), rectum, skin/subcutis and/or vagina. These lesions, which affected principally the arterial tree, were characterised as chronic endarteritis, periarterial oedema, arteriolar necrosis, arterial infarction, perinuclear chronic inflammation with eosinophils, phlebitis and chronic obliterative venous thrombosis.

MATERIALS AND METHODS

Study design

IL-4 was administered as a single intravenous bolus injection on day 1 followed by a 24-h continuous intravenous infusion on day 4 followed by daily subcutaneous injections over 2 weeks commencing on day 8. Treatment with IL-4 finished on day 21. Patients were admitted to hospital for 24-h observation on days 1, 4 and 8 and were further observed as outpatients on days 12, 15, 22, 29 and 36. The first, second, fifth and eighth subcutaneous injections (on days 8, 9, 12 and 15) were administered in hospital. The remaining subcutaneous injections were administered by a community nurse in the patients' homes. The study design was for 3 patients to be entered at each of the following dose levels: 40, 120, 280 and 400 $\mu g/m^2$/day. There was no dose escalation within individual patients. Patients who experienced a severe or life threatening toxicity (WHO grade 3 or 4) were to be withdrawn from treatment at that dose level. The study endpoint was a maximum tolerated dose (MTD) resulting in a WHO grade 3 or 4 toxicity in any system in 66% of patients. 6 patients were to be treated at the MTD. Once the MTD was reached, 3 further patients with low grade NHL were to be treated at a lower well tolerated dose of IL-4 because of specific interest in the response of this disease to treatment with IL-4.

Patients

19 patients were entered into this study. All had an advanced malignancy, 7 of whom had received prior treatment with chemotherapy alone and 1 patient had been treated with radiotherapy alone. Inclusion criteria were a Karnofsky performance status of 70% or more, age greater than 18 years, a minimum life expectancy of 3 months and a minimum period of 3 weeks (and to full recovery) following treatment with radiotherapy or chemotherapy (a minimum of 6 weeks for treatment with mitomycin C or nitrosureas). Although 3 patients with NHL had disease infiltration of their bone marrow, the remaining 16 patients had no evidence of bone marrow involvement. The patients had preserved renal function (serum creatinine ≤ 0.12 mmol/l and $\leq 2+$ proteinuria), preserved hepatic function (bilirubin ≤ 20 mmol/l and prothrombin time $\leq 1.3 \times$ control), preserved haemopoietic function (haemoglobin ≥ 10.0g/dl; WBC $\geq 3 \times 10^9$/l and platelet count $\geq 100 \times 10^9$/l) and negative stool guaic. Exclusion criteria included patients with a history of cardiac disease or with a New York Heart Association grade ≥ 2, patients with serious active infections or other serious intercurrent illness, a history of peptic ulceration or gastrointestinal bleeding, patients seropositive for HIV antigen, concomitant treatment with steroids or non-steroidal anti-inflammatory agents and fertile men or women unless using an acceptable method of contraception.

Clinical and laboratory monitoring

Patients were observed regularly during treatment and for 2 weeks following the final day of treatment with IL-4. Haematological and biochemical investigations were performed and included full blood counts (with white cell differential), measurement of prothrombin and partial thromboplastin times, full biochemical screen (with serum lactic dehydrogenase and gamma glutamyl transferase) and quantitative serum immunoglobulins (IgG, IgA and IgM). Serum CD 23 and IgE, peripheral blood lymphocyte phenotype, rhu IL-4 antibody assays, lymphocyte proliferative responses to various antigens, lymphocyte natural killing (NK) and lymphocyte activated killing (LAK) were also performed but these will be reported separately (Ghosh *et al*). Patients also had regular stool guaic tests. In addition, a bone marrow aspiration and biopsy was performed for microscopic morphological assessment pretreatment, immediately following treatment and 2 weeks after completion of treatment. The clinical state of the patients was monitored by clinical examination, recording of blood pressure, radial pulse, weight and oral temperature. In addition, a chest radiograph was performed pretreatment and 2 weeks after completion of therapy.

Pharmacokinetics

Pharmacokinetic studies were performed on day 1 [intravenous (i.v.) bolus], day 4/5 (24-h i.v. infusion), and day 8 (subcutaneous injection). Sample time points were as follows: (1) day 1–0, 5, 10, 15, 20, 30 and 45 min and 1, 1.5, 2, 4, 6 and 8 h; (2) day 4/5–0, 6 and 24 h; (3) day 8-0, 15, 30 and 45 min and 1, 2, 3, 4 and 8 h. Serum was assayed for pharmacokinetics using a sandwich type enzyme linked immunosorbent assay supplied by Sterling-Winthrop Group Ltd. Further details of the assay and the raw data obtained will be published separately (Ghosh *et al.*).

The data from the i.v. bolus was fitted to a one-compartment model using graphpad software. From the y axis intercept and the slope of the curve, the elimination half life and rate constant were determined. The area under the curve (AUC) was determined using the trapezoidal method. Total plasma clearance (Cl_{tot}) was derived from the formula: Cl_{tot} = dose/AUC and the apparent volume of distribution (V_d) was derived from the formula: $V_d = (Cl_{tot} \times t_{1/2})/0.693$ where $t_{1/2}$ is the elimination half life.

For the 24-h infusion and subcutaneous injection the AUC for each were determined using the trapezoidal method. The percentage bioavailability was determined by the equation: $[(AUC_{sc\ or\ inf})/AUC_{iv\ bolus})^*100]$.

Recombinant human IL-4

Recombinant human IL-4 was supplied by Sterling-Winthrop Group Ltd and was produced as a non-glycosylated protein of molecular weight 15.4 kD. Changes in the natural human IL-4 cDNA were made to promote the expression of rhu IL-4 as a homogeneous product. Rhu IL-4 differs from the natural cDNA

product at six residues. Aspartic acid is substituted for asparagine at positions 38 and 105 to preclude glycosylation; four amino acids (glutamine-alanine-glutamine-alanine) derived from a peptide leader used in the yeast expression system are not cleaved and remain at the amino terminus. The final product is formulated with sucrose, mannitol and TRIS and upon reconstitution with sterile water for injection has a pH of 7.5. It is supplied as a lyophilised preparation in vials containing 100 or 500 μg of rhu IL-4 (approximately 1.8×10^6 U/100 μg). The product is reconstituted with 0.5 or 1.0 ml of sterile water for injection, depending on the final concentration desired.

RESULTS

19 patients were entered into the study (see Table 1). 16 patients had solid tumours, including 8 cases of mesothelioma, 4 cases of non-small cell lung cancer (NSCLC), 3 cases of ovarian cancer and 1 case of leiomyosarcoma. Of these 16 patients, 3 patients each were treated with rhu IL-4 at 40 μg/m²/day and 120 μg/m²/day, 4 patients were treated at 280 μg/m²/day and 6 patients were treated at 400 μg/m²/day. We treated an additional 3 patients with NHL at the dose of 120 μg/m²/day because of specific interest in the response of this disease to treatment with rhu IL-4. All patients with NHL and ovarian cancer, and the single patient with leiomyosarcoma had received prior intensive treatment with chemotherapy and 1 patient with non-small cell lung cancer (NSCLC) had received prior treatment with radiotherapy. The remaining 11 patients had not been previously treated for their malignancy.

Toxicity

The majority (87%) of the adverse effects we observed as a consequence of treatment with IL-4 arose *de novo* in patients with no similar previous abnormality. Some patients had a pretreatment clinical complaint (e.g. lethargy) or biochemical abnormality (e.g. raised serum alkaline phosphatase) prior to commencing treatment. Thirteen percent of the reported adverse events in this phase I trial occurred in patients with a baseline abnormality which became more severe following treatment with IL-4. These latter events were given a WHO toxicity grading which equalled the difference between the baseline grading and the maximum grading achieved.

Table 1. Patients' characteristics

Patient no.	Diagnosis	IL-4 (μg/m²/day)	Completion of therapy	Response
1	NSCLC	40	Yes	Progressed
2	NSCLC	40	Yes	Static
3	NSCLC	40	Yes	Progressed
4	NSCLC	120	Yes	Static
5	Mesothelioma	120	Yes	Static
6	Mesothelioma	120	Yes	Static
7	NHL	120	Yes	Static*
8	NHL	120	Yes	Static*
9	NHL	120	Yes	Static†
10	Mesothelioma	280	Yes	Static
11	Ovarian carcinoma	280	Yes	Progressed
12	Ovarian carcinoma	280	No‡	Progressed
13	Ovarian carcinoma	280	Yes	Static
14	Sarcoma	400	No§	Progressed
15	Mesothelioma	400	No‡	Static
16	Mesothelioma	400	Yes	Static
17	Mesothelioma	400	Yes	Static
18	Mesothelioma	400	No‡	Static
19	Mesothelioma	400	No‡	Static

NSCLC = non-small cell lung cancer; NHL = low grade non-Hodgkin's lymphoma. * Transient poor partial response (less than 50%) during treatment with IL-4 but overall response was static. † Transient disease progression during treatment with IL-4 but overall response was static. ‡ Withdrawn from treatment after experiencing a WHO 3 or 4 toxicity. § Withdrawn from treatment as a result of disease progression.

Clinical toxicity was observed to be dose related. Patients treated at the higher dose levels of IL-4 experienced more severe symptomatology and a greater duration of symptomatology than patients treated at lower dose levels. In addition, patients became more symptomatic with cumulative exposure to rhu IL-4. Flu-like symptoms, gastrointestinal complaints, minor blood pressure changes and lethargy accounted for the majority of clinical toxicity (see Table 2). Other symptoms included 2 cases of subjective wheeze (in the absence of clinically detectable pulmonary bronchospasm or pulmonary oedema) and 1 case each of generalised pruritus and a vesicular rash. 1 patient became dyspnoeic following development of fluid retention but made a rapid and complete recovery following therapy with oral diuretics. 4 patients developed capillary leak syndrome which was manifested as either periorbital oedema or oedema of the hands and fingers. 1 patient developed a transient partial blindness of the right eye which lasted for 3 min. This patient's description of the visual defect corresponded to an upper nasal quadrantic hemianopia. On arrival at the clinic the patient's visual deficit had resolved and on examination his visual fields and acuity were found to be intact and fundoscopy was normal. This patient with NHL had a white cell count of 179×10^9/l and we concluded that vascular leucostasis was the probable cause of the patient's complaint, though perhaps aggravated by IL-4 enhancement of adhesion molecules as the patient had not had a similar complaint previously, despite equally high white cell counts. 1 patient complained of a "heavy feeling" in both lower limbs during part of the 24-h IL-4 infusion, however, there were no abnormal neurological findings.

The most serious clinical toxicities (WHO grade 3 or 4) occurred in patients being treated with IL-4 at 280 μg/m²/day and 400 μg/m²/day. 3 of 6 patients treated at 400 μg/m²/day were withdrawn prematurely from treatment as a direct result of toxicity. 1 patient was withdrawn from treatment on day 15 complaining of WHO grade III lethargy. The patient complained that it had taken him a full hour to get out of bed and get dressed that morning. On examination the patient was found to take up to 15 sec to start answering questions and his replies were slow and monotonous. His neurological and locomotor systems were found to be normal on clinical examination. A second patient was withdrawn on day 11 complaining of WHO grade III headache, lethargy, anorexia, nausea and vomiting. Both these patients demonstrated rapid resolution of toxicity within 24–48 h of stopping treatment with IL-4. A third patient was withdrawn from treatment on day 17 when he experienced a small haematemesis. The patient's haemodynamic status and haemoglobin readings remained stable following the haematemesis and there was no further recurrence of the event. The patient admitted to nose bleeds prior to the haematemesis and it is plausible that he may have swallowed some blood which he later vomited. An upper gastrointestinal endoscopic examination was performed to the level of the first part of the duodenum and no abnormality was found.

The most serious toxicity of the trial occurred in a patient being treated with IL-4 at a dose of 280 μg/m²/day. The patient

Table 2. Clinical toxicity

	40 μg/m²/day 3 patients No. patients/ max. WHO				120 μg/m²/day 6 patients No. patients/ max. WHO				280 μg/m²/day 4 patients No. patients/ max. WHO				400 μg/m²/day 6 patients No. patients/ max. WHO			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Fever	1	2			4				2	2			1	4		
Flushed		2			1				1				3			
Chills		1			2	1								1		
Rigors						1								2		
Sweats		1												2		
Rhinitis						1				1			1	2		
Conjunctivitis														1		
Headache		1				2				1				2	1	
Cough						1			1							
Epigastric discomfort						1					1		1	2		
Anorexia														1	1	
Dysphagia									1							
Nausea and vomiting					1	3			1					3	1	
Diarrhoea					2									2		
Constipation													1			
Flatulence						1										
Abdominal colic											1			2		
Gastrointestinal bleeding												1			1	
Decreased blood pressure	3				5				2	1			3			
Increased blood pressure	1				4				2				5			
Lethargy	1	1			2				1				2		2	
Wheeze																
Fluid retention										1						
Capillary leak syndrome						1				1				2		
Pruritis										1				1		
Vesicular rash														1		
Visual field deficit						1										

presented on day 19 of treatment with a 24-h history of two large haematemeses and one episode of malaena. On examination she was found to be hypotensive (blood pressure 80/50 mmHg standing) and anaemic (the haemoglobin having fallen from 13.3 to 10.8 g/dl over 4 days). She was immediately resuscitated and transfused with four units of whole blood. Endoscopy revealed an extensive haemorrhagic oesophagitis. The patient had no prior clinical history to explain the finding and she had not been taking non-steroidal anti-inflammatory agents. Treatment with IL-4 was stopped and the patient was commenced on treatment with antacids and a histamine-2-receptor antagonist. The patient had no further recurrence of bleeding. The remaining 3 patients treated with IL-4 at a dose of 280 μg/m²/day all completed therapy, though 1 patient developed unexplained transient abdominal pain. All patients treated with IL-4 at the lower doses of 120 μg/m²/day and 40 μg/m²/day completed therapy and with no record of severe clinical toxicity.

Biochemical toxicity was limited to asymptomatic elevation of liver enzymes (see Table 3). During treatment with IL-4 there was WHO grade 1 or greater elevation of serum alkaline phosphatase (13 patients), gamma glutamyltransferase (2 patients), aspartate transamine (3 patients) and lactate dehydrogenase (2 patients). There were 2 cases of WHO grade 3 biochemical toxicity, both with respect to elevation of alkaline phosphatase and both occurring at an IL-4 dose of 400 μg/m²/day. The time relationship between serum alkaline phosphatase and treatment with IL-4 is shown in Fig. 1. There were no consistent changes in serum bilirubin, immunoglobulins, proteins and electrolytes. IL-4-induced liver damage was not accompanied by coagulation abnormalities. Only 3 patients developed prolongation of prothrombin time (PT) or partial thromboplastin time (PTT) and all were WHO grade 1 and not dose related. 3 patients developed WHO grade 1 or 2 anaemia which were also not dose related. A single patient treated at 400 μg/m²/day developed a WHO grade

Table 3. Biochemical and haematological toxicity

	40 μg/m²/day 3 patients No. patients/ max. WHO				120 μg/m²/day 6 patients No. patients/ max. WHO				280 μg/m²/day 4 patients No. patients/ max. WHO				400 μg/m²/day 6 patients No. patients/ max. WHO			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Increased alkaline phosphatase	1	2				3				2	1			3	2	
Increased LDH													1	1		
Increased AST						2				1						
Increased GGT													1	1		
Anaemia	1	1				1										
Increased PT	1															
Increased PTT					1				1							
Thrombocytopenia															1	

LDH = lactate dehydrogenase; AST = aspartate aminotransferase; GGT = gamma glutamyltransferase; PT = prothrombin time; PTT = partial thromboplastin time.

3 thrombocytopenia. Other patients developed fluctuations in haemoglobin and platelet counts which did not reach WHO grade toxicity and which were not clearly related to treatment with IL-4. There was no leucocyte WHO toxicity. Several patients developed elevation of leucocyte populations, including neutrophils (3 patients), lymphocytes (1 patient), monocytes (5 patients), eosinophils (3 patients) and basophils (1 patient).

Bone marrow responses

10 of the 19 patients entered into this phase I trial of IL-4 developed bone marrow changes following treatment (see Table 4). These changes were very variable though were noted to occur more frequently at higher dose levels. Observations included lymphocytosis, eosinophilia and increases in plasma cells, erythroid cells and megakaryocytes and an increased myeloid/erythroid ratio. Several patients developed several of these changes.

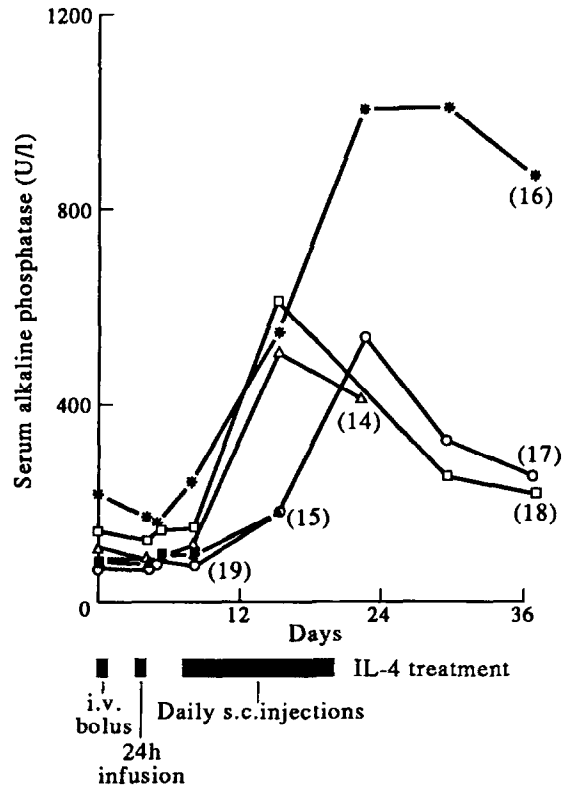

Fig. 1. Serum alkaline phosphatase vs. time plot for the 6 patients receiving IL-4 at 400 μg/m². Patient trial numbers indicated in parentheses.

Tumour responses

All 19 patients entered into this study had advanced metastatic disease. There were no responses among 16 patients with solid tumours and 5 of these patients had progression of their disease while on treatment with IL-4. 2 of the 3 patients with low grade NHL had brief minor responses following initiation of treatment with IL-4 but both relapsed prior to the completion of their therapy. 1 of these patients with NHL had a brief fall in his circulating lymphoma cell count from 174 to 102 × 10^9/l. This patient also experienced an improvement in his night sweats. Another patient with NHL had a brief drop in his circulating lymphoma cell count from 8.63 to 1.63 × 10^9/l. The third patient with NHL had a transient increase in the size of his lymphadenopathy and abdominal organomegaly following initiation of treatment with IL-4 but the measurements had returned to baseline prior to completion of therapy.

Pharmacokinetics

The pharmacokinetic parameters were determined in a total of 12 patients at all four dose levels. The data for the i.v. bolus best fitted a one compartment model and this data is summarised in Table 5. There is a linear relationship between IL-4 dose and IL-4 AUC indicating linear pharmacokinetics over the dose range studied (Fig. 2). The volume of distribution is small

Table 4. Bone marrow changes

Bone marrow appearances	No. patients
Eosinophilia	4
Lymphocytosis	4
Increased plasma cells	2
Increased M:E ratio	2
Increased megakaryocytes	1

M:E ratio = myeloid erythroid ratio.

Table 5. Pharmacokinetic parameters for the intravenous bolus injection route

Dose IL-4 (μg/m²/day)	AUC (ng/ml/h)	$t_{1/2}$ (min)	Cl_{tot} (ml/min)	V_d (l)
40	5.76	15.69	202.63	4.65
120	22.34	20.04	240.06	7.17
280	74.21	19.39	153.03	4.24
400	108.95	22.13	108.80	3.41

AUC = area under curve; $t_{1/2}$ = elimination half life; Cl_{tot} = total plasma clearance; V_d = apparent volume of distribution.

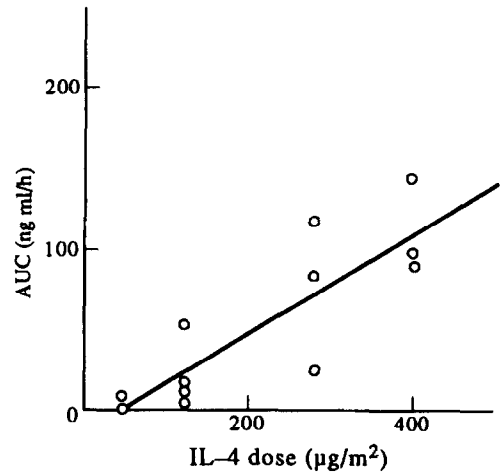

Fig. 2. Relationship between dose of IL-4 and AUC (concentration/time curve) following intravenous bolus injection.

(mean V_d = 4.9 ± 3.68 l). The half life is short (mean $t_{1/2}$ = 19 ± 8.7 min) indicating a rapid clearance from the circulation. A typical plasma concentration vs. time curve is shown in Fig. 3.

The mean bioavailabilities for IL-4 for the 24-h intravenous infusion ranged from 34 to 71% and for the subcutaneous injection from 34 to 62% and are summarised in Table 6.

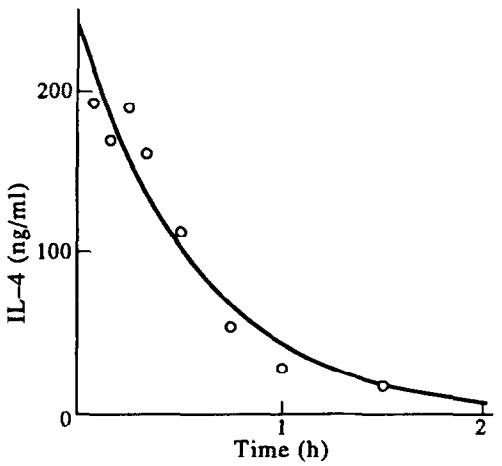

Fig. 3. Concentration of IL-4 in the serum over time for 1 patient receiving IL-4 at 400 μg/m² as an intravenous bolus injection.

DISCUSSION

This phase I study of rhu IL-4 in 19 patients has examined the toxicity produced when rhu IL-4 is given by a single intravenous bolus injection, a single 24-h intravenous infusion and 2 weeks of daily subcutaneous injections. In addition, the pharmacokinetics of IL-4 have been investigated following the different routes of administration.

Gilleece *et al.* have published the results of a phase I toxicity study of *E. coli*-derived IL-4 administered by subcutaneous injection at daily doses of 0.5, 1.0 or 5.0 μg/kg to 9 patients [26]. Dose-limiting toxicity was reached at 5 μg/kg/day. Symptoms of toxicity included fatigue, flu-like symptoms and elevated liver enzymes. Modest but significant elevations of neutrophil and platelet counts occurred. No clear evidence of antitumour effects emerged although pain in metastatic lymph nodes and a small fall in myeloma paraprotein levels during dosing were observed.

Preliminary results from five other phase I trials with rhu IL-4 have been published in abstract format [22, 27–30]. Three studies [27–29] were conducted with patients who had solid tumours and using yeast-derived IL-4. A further two studies [22, 30] were carried out using *E. coli*-derived IL-4 and included patients with either solid tumours or haematological malignancies. Clinical side-effects included flu-like symptoms, gastrointestinal upset, fatigue, fluid retention and gastric ulceration. Patients who developed gastric ulceration were also receiving concomitant treatment with non-steroidal anti-inflammatory agents which may have been contributory. No allergic manifestations have been described. Biochemical toxicity included elevation of liver enzymes in most studies and renal dysfunction in one study. Slight but consistent prolongation of PT and PTT was observed in one study. A single patient with renal cancer developed a transient mixed tumour response to treatment with yeast-derived IL-4 but later progressed before completion of treatment. A trial with *E. coli*-derived IL-4 noted a rapid shrinkage of lymph nodes and spleen in a patient with low grade NHL and a fall in circulating malignant B cells from 18 × 10^9/l to 5 × 10^9/l within 6 h of a single subcutaneous bolus injection in a patient with chronic lymphocytic leukaemia. Limited pharmacokinetic data are available from two studies. Yeast-derived IL-4 was detectable in the serum at time points up to 12 h after subcutaneous administration. *E. coli*-derived IL-4 peaked in the serum at 4–6 h following injection and was detectable for up to 8 h. No pharmacokinetic data are available following i.v. administration of IL-4.

The predominant clinical side-effects observed in our study included flu-like symptoms, gastrointestinal upset, lethargy and

Table 6. Bioavailability for the 24-h intravenous infusion and subcutaneous injection rates

Dose IL-4 (μg/m²/day)	% bioavailability (s.c. injection) Mean	S.D.	% bioavailability (24-h i.v. infusion) Mean	S.D.
40	0	0	0	0
120	63	76	62	77
280	34	28	36	45
400	71	14	34	34

s.c. = subcutaneous; i.v. = intravenous.

transient hypotension. Several features were similar to those seen following treatment with a variety of other recombinant cytokines and similar to those reported in preliminary results of other IL-4 phase I trials. In addition, there were several cases of capillary leak syndrome, a case of life-threatening haemorrhagic oesophagitis, a second case of unexplained gastrointestinal bleeding and single individual cases of fluid retention, vesicular rash, generalised pruritus and transient visual field deficit. Capillary leak syndrome and fluid retention have also been reported following treatment with IL-4. Gastritis with ulceration was reported from one phase I trial but these patients were also taking non-steroidal anti-inflammatory agents. Considering the histomorphological findings previously described in cynomolgous monkeys, it is possible that our case of acute haemorrhagic oesophagitis, and the other reported cases of gastritis and gastric ulceration, are a consequence of IL-4-induced vasculitis. The single case of transient upper nasal quadrantic haemianopia was almost certainly due to leucostasis in a patient with a high peripheral lymphoma cell count but perhaps precipitated by IL-4-induced expression of cell surface adhesion molecules.

Biochemical toxicity was limited to asymptomatic elevation of liver enzymes suggesting IL-4-induced liver damage. Alkaline phosphatase isoenzyme studies were not carried out in our study but in a previous study where 1 patient only was examined, the serum enzyme elevation was shown to be of liver origin. An hepatic origin for the observed rise in serum alkaline phosphatase is in keeping with the other liver enzymes also shown to be elevated in the serum following treatment with IL-4. This is similar to findings in cynomolgous monkeys treated with IL-4 and preliminary data of other IL-4 phase I clinical trials. There were no consistent changes in serum bilirubin, electrolytes, proteins and immunoglobulins and no significant prolongation of prothrombin or partial thromboplastin times. No clear consistent haematological toxicity was seen. Several patients developed elevations of individual leucocyte populations, including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Histological observation of bone marrow taken during and after treatment revealed very variable findings though lymphocytosis and eosinophilia was observed in 4 patients each, respectively.

All patients experienced some toxicity but at IL-4 doses of 40 and 120 $\mu g/m^2$/day these events were of maximum WHO grade 2 and did not interfere with dosing. The single case of haemorrhagic oesophagitis represented the most serious toxicity of the trial and occurred in 1 of 4 patients treated at 280 $\mu g/m^2$/day. The 3 other patients treated at 280 $\mu g/m^2$/day successfully completed treatment, though 1 experienced unexplained and transient WHO grade 3 abdominal discomfort. The MTD of IL-4 administered by the intravenous and subcutaneous routes described is 400 $\mu g/m^2$/day. 3 of the 6 patients treated at this dose level were withdrawn from treatment as a result of IL-4-induced toxicity.

The observation of a significant decrease in the circulating lymphoma cell count of 2 patients with previously treated NHL was very interesting. However, both responses were transient and reversed before the completion of treatment. This is comparable with the preliminary report from another phase I trial of a rapid transient decrease in circulating malignant B cells in a patient with chronic lymphocytic leukaemia (CLL) and the rapid transient shrinkage of lymph nodes and spleen in a patient with low grade NHL within hours of a single subcutaneous injection of IL-4. The third patient with NHL treated in our trial experienced brief disease progression with an increase in the size of his peripheral lymphadenopathy and abdominal organomegaly, but these changes also reversed before the completion of treatment. There was no clear evidence of a response to treatment with rhu IL-4 in 16 patients with solid tumours.

The pharmacokinetics of IL-4 following intravenous bolus administration are characterised by a rapid clearance from the systemic circulation. The short half life is consistent with that seen for many other cytokines. The rapid clearance of IL-4 with the low volume of distribution might indicate rapid and relatively irreversible binding to IL-4 receptors on peripheral blood cells leading to retention of the drug within the systemic circulation, albeit in a compartment where the drug cannot be assayed.

The bioavailability of IL-4 showed significant variability following both 24-h i.v. infusion and subcutaneous administration. These data must be interpreted with some caution because the time course of IL-4 elimination was only followed out to 8 h in the subcutaneous infusion studies and only three time points were studied in the 24-h infusion studies. Thus, the true bioavailability might be higher with these alternative routes of administration.

Human recombinant interleukin-4 (rhu IL-4) is a multifunctional cytokine with pleiotropic effects on the immune, haemopoietic and endothelial systems. Results of preclinical studies suggest a potential therapeutic role for this cytokine as an anticancer agent. However, *in vivo* animal studies have revealed that IL-4 produces potentially serious vasculitis affecting many tissues, including the gastrointestinal tract and heart. Our single case of severe haemorrhagic oesophagitis and the report of gastritis and gastric ulceration in another phase I trial may be a manifestation of this. We conclude though that there is sufficient preclinical evidence to justify the use of rhu IL-4 in phase II trials for patients with lymphoid malignancies.

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Eur J Cancer, Vol. 29A, No. 12, pp. 1707–1711, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00
Pergamon Press Ltd

Patient Acceptability and Practical Implications of Pharmacokinetic Studies in Patients With Advanced Cancer

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We have studied the practical implications and acceptability to patients of pharmacokinetic studies in 34 women receiving anthracyclines for advanced breast cancer. The following parameters were recorded: age, ECOG performance status, psychological state (Rotterdam Symptom Checklist), cytotoxic drug and dose, number of venepunctures for treatment and sampling, and time when the sampling cannula was removed. Immediately after finishing pharmacokinetic sampling, patients completed a questionnaire which revealed that (i) all patients understood sampling was for research, (ii) 35% of patients experienced problems with sampling, (iii) benefits from participation were perceived by 56% of patients. Of 20 patients later questioned after completion of their treatment course, 40% recalled difficulties with blood sampling. Factors identifying in advance those patients who tolerate pharmacokinetic studies poorly were not identified but the number of venepunctures should be minimised. Patients may also perceive benefits from 'non-therapeutic' research.
Eur J Cancer, Vol. 29A, No. 12, pp. 1707–1711, 1993.

INTRODUCTION

BY THEIR very nature, cytotoxic drugs cannot be studied in healthy volunteers, and species differences in drug distribution and metabolism limit the relevance of animal studies. Pharmacokinetic studies have an important place in phase I [1, 2] and phase II [3] trials of cytotoxic agents. In clinical practice the optimal use of carboplatin [4] and etoposide [5] has been influenced by the results of pharmacokinetic studies. These studies are, therefore, essential for the optimal development and use of cytotoxic drugs.

The ethical and practical issues raised by clinical pharmacokinetic studies were discussed by Svensson [6]. These consider-