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Original Paper

Clinical Effects of Human Macrophage Inflammatory Protein-1 Alpha MIP-1α (LD78) Administration to Humans: a Phase I Study in Cancer Patients and Normal Healthy Volunteers with the Genetically Engineered Variant, BB-10010

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BB-10010 is a genetically engineered variant of human macrophage inflammatory protein-1 alpha (hMIP-1α) with improved pharmaceutical formulation properties. Although initially described as a pro-inflammatory cytokine, it is now recognised that hMIP-1α has additional effects on haemopoietic stem cell cycling and on human immunodeficiency virus uptake by macrophages. In view of the potential clinical utility of the molecule, we have embarked on a clinical trials programme to evaluate the safety, tolerability and haematological effects of BB-10010. We now report the results of two phase I clinical studies in which 49 subjects (9 patients with advanced breast carcinoma and 40 normal healthy volunteers) received escalating doses of BB-10010, from 0.1 to 300 μg/kg using the subcutaneous (s.c.) or intravenous route (i.v.) of administration. Treatment was associated with a dose-related increase in monocyte count which peaked at 200% of steady-state levels and was preceded by an acute, short-lived, monocytopenia, 50–100% of baseline. No measurable effects were noted on other leucocyte subsets or on circulating progenitor cell numbers. In all cases, BB-10010 was extremely well tolerated with no significant toxicity observed at any dose level and a maximum tolerated dose was not defined. Pharmacokinetic analysis revealed that serum concentrations of BB-10010 were detectable using doses of ≥ 10 μg/kg i.v. or ≥ 30 μg/kg s.c., and that a single s.c. injection resulted in sustained plasma levels over a 24 h period. These preliminary studies have confirmed the safety and tolerability of BB-10010 using a dose range up to 300 μg/kg. Further clinical studies are ongoing to determine the biological effects and to investigate the potential myeloprotective properties using a variable dose range and schedule of BB-10010 in combination with cytotoxic chemotherapy. © **1998 Elsevier Science Ltd. All rights reserved**

Key words: BB-10010, MIP-1α, phase I study, mobilisation, stem cell, LD78
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INTRODUCTION

HUMAN MACROPHAGE inflammatory protein-1 alpha (hMIP-1α) is a member of the C–C chemokine family of molecules. These small, structurally related and multifunctional peptides are thought to possess pro-inflammatory and reparative activity based on *in vitro* studies [1, 2]. In addition to the proposed role during inflammation, hMIP-1α acts as a specific inhibitor of haemopoietic stem cell proliferation [3, 4]. This latter property has been exploited in a number of experimental chemotherapy models and may provide a novel mechanism for protecting haemopoietic stem cells against the

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myelosuppressive effects of cancer chemotherapy [4, 5]. In addition to the cell-cycle inhibitory properties, hMIP-1α also induces an acute mobilisation of murine bone marrow progenitor cells with a peak effect within 30 min of administration [6]. More recently, hMIP-1α was identified (along with MIP-1β and RANTES which are also in the class of C–C chemokines) as one of the major HIV-suppressor factors produced by $CD8^+$ T cells [7, 8]. The mechanism for this inhibition was clarified when the C–C chemokine receptor CCR5 was found to be a co-receptor for primary M-tropic HIV-1 strains [9]. This latter property suggests that MIP-1α may be able to break the cycle of infection in HIV-1 seropositive individuals.

Clinical investigation of hMIP-1α has been hampered by the tendency of the wild-type molecule (mol. wt. 7712 Da) to undergo extensive self-association. Under physiological conditions this leads to the formation of heterogeneous, high molecular weight aggregrates (mol. wt. > 100 000 Da) which result in considerable problems with drug formulation and production. BB-10010 represents a genetically engineered variant of hMIP-1α that carries a single amino acid substitution of Asp⇒Ala at position 26 in the primary sequence [10]. This variant has a greatly reduced tendency to polymerise, yet it still retains hMIP-1α-like agonist activity in receptor binding, calcium mobilisation, inhibition of colony formation and thymidine suicide assays [10]. We have recently shown that BB-10010 is active *in vivo*, both as a myeloprotectant [11] and by inducing a neutrophilia in mice accompanied by mobilisation of haemopoietic progenitor cells from the bone marrow to peripheral blood [6, 12].

The combined myeloprotective and stem cell mobilising properties of BB-10010 suggested that the molecule might play an important therapeutic role in ameliorating chemotherapy-induced bone marrow damage, while enhancing peripheral blood stem cell mobilisation for transplantation purposes. Here we report the results of two phase I studies, one in cancer patients and one in normal healthy volunteers. In view of the nature of the molecule and safety data from detailed toxicology studies (no significant toxicity up to 10 mg/kg, British Biotech Pharmaceuticals Ltd) we did not anticipate that determination of a maximum tolerated dose would be a realistic endpoint. The dose range selected for initial investigation was, therefore, based on *in vitro* data that showed receptor occupancy and biological responses with BB-10010 concentrations of 10–150 ng/ml. Furthermore, *in vivo* chemotherapy protection models in rodents revealed activity using a dose range of 100–500 μg/kg [3–6, 11] and translating data on other haemopoietic cytokines, albeit growth factors not inhibitors, it is appears likely that the active dose range in humans may be much lower than that required in rodents. The objectives of these first studies were, therefore, to assess the biological effects, safety, tolerance and pharmacokinetics of BB-10010 using a dose range of 0.1–300 μg/kg, and to compare three modes of administration (subcutaneous (s.c.) infusion, s.c. or intravenous (i.v.) bolus) in normal subjects and in cancer patients with normal haemopoiesis in the absence of chemotherapy. This is the first report of administration of a C–C chemokine in human subjects.

PATIENTS AND METHODS

Study design

Two separate phase I studies in normal healthy male volunteers and advanced breast cancer patients were carried out between January 1995 and October 1995. Both studies received full ethical approval.

Health volunteer study. This was a randomised, placebo controlled, dose ranging study of the effects of single doses of i.v. and s.c. BB-10010 in a total of 40 healthy male volunteers aged 20–38 years. Screened subjects were required to have a normal haematology and biochemical profile and underwent a full physical examination, vital signs, electrocardiogram and assessment for anti-BB-10010 antibodies. The study was conducted by Hammersmith Medical Research at the Central Middlesex Hospital, London, U.K. and written informed consent was obtained from all subjects.

Subjects received one dose only and at each dose level, three received BB-10010 and one placebo. Higher doses were administered only when the previous lower dose was shown to be well tolerated. Five dose levels of BB-10010 were given either as a s.c. or i.v. bolus injection. Ten groups received BB-10010 in total with i.v. dose levels of 0.1, 1, 10, 30 or 100 μg/kg or s.c. dose levels of 1, 10, 30, 100 or 300 μg/kg.

Cancer patient study. This was an open, dose escalating study of BB-10010 administered s.c. to 9 female patients with advanced (stage IV) breast cancer, carried out at the Christie Hospital, Manchester, U.K. Groups of 3 subjects received a s.c. bolus injection of 1, 10 or 100 μg/kg BB-10010 followed 1 week later by the same dose, but this time infused s.c. over a 24 h period.

The median age was 52 years (range 43–75 years) with WHO performance status ≤ 1. All were stable on no treatment or endocrine therapy only. Inclusion criteria were: histologically proven cancer, normal haemopoiesis as judged by a white blood cell count > 3000 cells/mm^3, platelets > 100 000 cells/mm^3 and haemoglobin > 10 g/dl, 18 years or older, bilirubin < 2× upper limit of normal, stable hormonal therapy for at least 3 months and a predicted survival > 3 months. Exclusion criteria were: history of life-threatening anaphylactic reaction, acute illness within 2 weeks of the study, the use of other investigational agents during the study (these included granulocyte colony stimulating factor (G-CSF) and/or other cytokines), concomitant treatment with steroids, pregnant or breast-feeding females and any condition which in the opinion of the investigator might make the patient unsuitable for the study. Written informed consent was obtained from all subjects before entrance to the study.

Clinical and laboratory monitoring

All subjects underwent initial baseline screening tests including routine haematology (full blood count (FBC) and differential), biochemical profile (including liver function tests, renal function, blood glucose and lactate dehydrogenase (LDH)) and electrocardiography. During the study period, haematological parameters were intensively monitored and were determined using an automated counter (Coulter) and confirmed by manual differential count. Measurement of circulating progenitor cells and development of anti-BB-10010 antibodies are described in detail below. The clinical state of the subjects was monitored by regular physical examination, recording of blood pressure, radial pulse and oral temperature. In addition, the BB-10010 injection site was inspected regularly for signs of a local inflammatory response.

Clonogenic assays. Clonogenic progenitor cells were assayed at frequent time intervals in the peripheral blood, collected 30 min to 24 h after bolus injection of BB-10010. Mononuclear cells were separated using a Ficol Hypaque

gradient (density 1.077 g/ml, Pharmacia, Germany). Mononuclear cells were washed twice in phosphate buffered saline (PBS) supplemented with 2% fetal calf serum (FCS) and counted in a Neubauer haemocytometer.

Iscove's modified Dulbecco's medium (IMDM, Gibco, Paisley, U.K.) was supplemented with 4×10^{-3} M glutamine, 10^{-7} M sodium selenite, 2.5×10^{-4} M alpha thioglycerol, 30% pretested FCS, 10% medium conditioned by the cell line 5637 as a source of growth factors, 1% de-ionised bovine serum albumin (BSA) (Sigma Chemical Co., Poole, Dorset, U.K.) and 2 units of recombinant erythropoietin (Epo) (Boehringer Mannheim U.K. Ltd, Sussex, U.K.) per ml of culture. Cells were cultured in 1.35% methylcellulose with supplemented IMDM in 24 well standard tissue culture plates (Falcon, Runcorn, U.K.) in triplicate to a concentration of 1×10^5 mononuclear cells/ml. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 5% O_2 in nitrogen. Colonies were counted and classified after 14 days' growth as granulocyte–macrophage colony forming cell (GM-CFC), burst-forming unit-erythroid (BFU-E), or multipotent (Mix-CFC) progenitor cells, as previously described [13].

Determination of CD34 positive cells. Mononuclear cells were washed twice in saline supplemented with 1% BSA and incubated with a mouse anti-CD34 fluorescein isothiocyanate conjugated monoclonal antibody (anti-HPCA-2, Becton-Dickinson Oxford, U.K.) for 30 min at 4°C. Cells were washed twice in saline supplemented with 3% BSA. Cells were stored in a fixative of 1% formaldehyde solution and were analysed on a fluorescent activated cell sorter (FACS) within 24 h. A non-specific isotype matched fluorescein isothiocyanate conjugated monoclonal antibody was used as a control [14].

Pharmacokinetics

Blood samples (3 ml) were collected in sodium citrate at regular time intervals between 15 min and 48 h after injection of BB-10010. Samples were centrifuged at 3000 ***g*** for 10 min at 4°C and the supernatent frozen at − 20°C until analysed. Samples were subsequently assayed by enzyme linked immunoabsorbant assay (ELISA) for BB-10010 using the Quantikine™ kit (R&D Systems Inc. Abingdon, U.K.). The assay was validated for the determination of BB-10010 over the range 47–1500 pg/ml. The lower limit of quantification was 93.8 pg/ml.

BB-10010 *antibody determination*

BB-10010 antibodies were determined using an anti-BB-10010 ELISA at screening, day 28 and day 42 in the volunteers and at day 0, 7 and 14 in the advanced cancer study. Briefly, sterile plates (Gibco) were sensitised with sodium carbonate/bicarbonate, pH 9.6 overnight at 4°C with BB-10010. The wells were then washed and test sera added in duplicate at 1:250 dilution and serum standards at 1:4000–1:256 000 dilutions. After incubating the primary antibody (Macaque anti-LD78 serum) for 2 h at 4°C, the wells were washed and peroxidase-labelled antihuman IgG (Sigma) added. After further washing, 0.01% 3,3′,5,5′-tetramethylbenzidine (TMB) was added and the reaction was allowed to proceed for 5 min before stopping with sulphuric acid. Results were read at 450 nm and data expressed as antibody units derived from the standard curve. Curve fitting and data reduction were done using a Denley ELISA + software package.

BB-10010

BB-10010 was supplied by British Biotech Pharmaceuticals Ltd as a sterile solution at 2 mg/ml concentration. Ampoules of drug were stored at − 20°C then thawed and diluted with normal saline to the appropriate concentration immediately prior to administration. All drug handling was carried out under strict aseptic conditions in a microbiological safety cabinet.

RESULTS

Safety

BB-10010 was extremely well tolerated, such that no maximum tolerated dose (MTD) was defined in either study. In the volunteer study, no serious adverse events were noted. 13 of 30 subjects who received BB-10010 and 1 of 10 who received placebo reported cough and rhinitis. No clinically significant local or systemic inflammatory response was observed, but several subjects reported slight pain and minor redness at the injection site. The incidence of this was not dose related and, overall, the incidence of adverse events was similar after placebo and i.v. BB-10010 (6/10 and 8/15, respectively) while the incidence after s.c. BB-10010 (4/15) was somewhat lower than after placebo.

In the 9 cancer patients, BB-10010 was well tolerated after both s.c. bolus and infusion. Only 4 of 8 adverse events reported were considered to be possibly related to BB-10010 administration. These were headache in 1 subject and flushing in another after infusion of 1 μg/kg, dizziness in 1 subject after bolus injection and infusion of 10 μg/kg and dizziness in 1 subject after infusion of 100 μg/kg. Dizziness was not associated with any changes in haemodynamic parameters in either subject. No allergic or anaphylactic reactions occurred despite the use of up to 20 mg of protein. There was no significant fluctuation of antibody generation throughout the period of either study and no clinically significant alteration in the biochemical indices, hepatic or renal function was noted at any dose level.

Haematological response

The haematological response to BB-10010 was determined by measuring changes in the total and differential leucocyte count and circulating numbers of colony-forming cells. Neither i.v. nor s.c. bolus injection of BB-10010 produced a measurable effect on the total white blood cell count or on leucocyte subsets, including neutrophils, eosinophils, lymphocytes or basophils (results not shown). However, a modest monocytosis was observed in both cancer patients and normal volunteers following bolus injection, which was dose dependent following s.c. administration. A maximal increase of 200% of baseline levels (Figure 1) was observed at differing time points depending on the mode of administration. After s.c. bolus injection, BB-10010 resulted in a sustained increase in monocyte numbers over at least 12 h (Figure 1a), preceded by a transient monocytopenia of 50–100% of baseline. A monocytosis was also evident following i.v. bolus injection, but the kinetics differed with an early peak at around 1–2 h and normalisation by 4 h (Figure 1b).

The monocyte response following both i.v. and s.c. BB-10010 was mirrored by the respective BB-10010 pharmacokinetics (see below).

The total circulating progenitor cell (CFCs) numbers showed a maximal 3-fold increase over baseline levels at 24 h (10 μg/kg s.c bolus) following BB-10010, but no clear dose

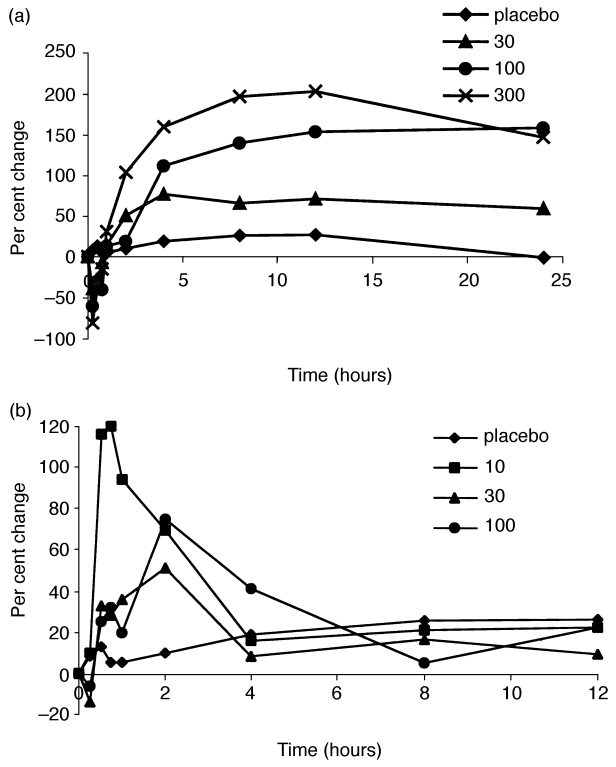

Figure 1. Mean percentage change in monocyte counts of healthy volunteers following a single administration of (a) subcutaneous BB-10010 (30, 100 and 300 μg/kg), (b) intravenous BB-10010 (10, 30 and 100 μg/kg).

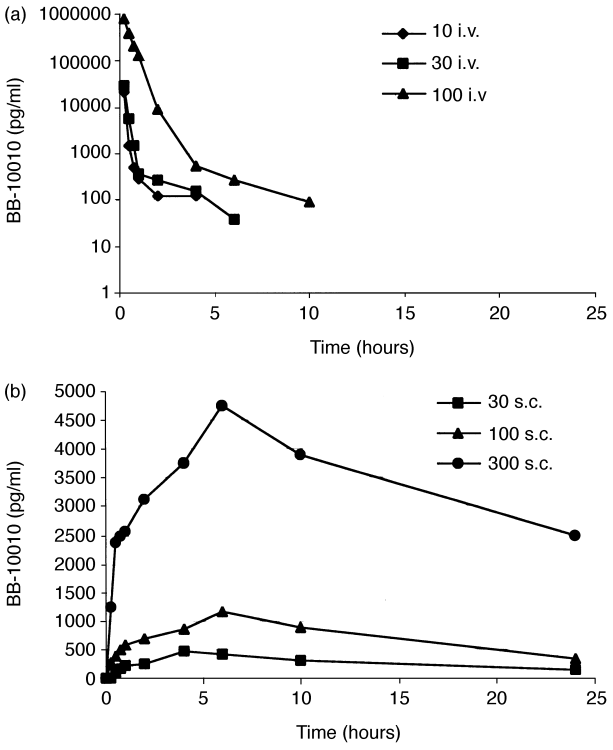

Figure 2. Plasma levels of BB-10010 following (a) 10, 30 and 100 μg/kg intravenous injection and (b) 30, 100 and 300 μg/kg subcutaneous injection in normal healthy volunteers.

response nor consistent pattern of mobilisation was observed (Table 1). No effect was noted on the GM-CFC population or $CD34^+$ cell population (results not shown).

BB-10010 *pharmacokinetics*

The plasma concentration of BB-10010 following i.v. and s.c. bolus administration in normal volunteers is shown in Figure 2. Intravenous injection was associated with an initial high peak followed by a rapid decline. The maximum concentrations (C_{max}) after 10, 30 and 100 μg/kg were 19 700, 55 700 and 787 000 pg/ml, all occurring by 0.25 h, the terminal half-life ranging from 1.7 h at the lower dose level to 2.8 h at the 100 μg/kg dose level (Table 2). BB-10010 was not quantifiable in any subject beyond 12 h following i.v. dosing. Area under the curve (AUC) results showed a non-linear increase with increasing dose with a mean of 3540 pg/ml/h at 10 μg/kg, 17 600 pg/ml/h at 30 μg/kg and 326 000 pg/ml/h at 100 μg/kg.

Subcutaneous injection resulted in a more favourable pharmacokinetic profile with a sustained plasma level over the 24 h period (Table 2). Mean concentrations following 30, 100 and 300 μg/kg BB-10010 at 24 h were 134, 327 and 2497 pg/ml, respectively. C_{max} values ranged from 137 pg/ml at the 10 μg/kg dose to 5590 pg/ml following 300 μg/kg, the peaks achieved between 4.3 and 6.67 h (T_{max}).

In the cancer patients, BB-10010 was not generally detectable in plasma after bolus or infused doses of 1 or 10 μg/kg. The mean C_{max} after bolus and infused doses of 100 μg/kg were 2645 ± 1813 and 850 ± 152 pg/ml with the corresponding AUC being $41\,300 \pm 16\,200$ (pg/h)/ml and 1450 ± 91 (pg/day/ml). The terminal elimination half-lives were 14.4 ± 5.7 h and 0.6 ± 0.2 days, respectively. Subcutaneous infusion of BB-10010 (100 μg/kg) produced a constant plasma level from 4 to 36 h with mean plasma levels

Table 1. *Total colony-forming cells in peripheral blood following BB*-10010

Dose (μg/kg)	Mode of administration	Time (h)					Number
		0	1	2	4	24	
1	s.c. bolus	1.0	0.9	0.8	0.6	1.0	3
10	s.c. bolus	1.0	1.2	1.4	2.2	3.0	3
30	i.v. bolus	1.0	1.1	1.5	0.5	ND	3
100	s.c. bolus	1.0	0.9	0.9	1.1	0.5	6
100	s.c. infusion	1.0	ND	ND	1.6	1.1	3
Placebo	i.v. bolus	1.0	1.0	0.7	0.9	0.7	3
All BB-10010		1.0	1.0	1.0	1.2	1.4	18

BB-10010 was administered as a subcutaneous (s.c.) or intravenous (i.v.) bolus or 24 h s.c. infusion. Results show the relative change from baseline (mean values) for each group and all groups combined. ND, not done.

Table 2. Pharmacokinetics following BB-10010 bolus injection in healthy volunteers

	Intravenous BB-10010 (μg/kg)				Subcutaneous BB-10010 (μg/kg)			
	1 ($n=3$)	10 ($n=3$)	30 ($n=3$)	100 ($n=3$)	10 ($n=1$)	30 ($n=3$)	100 ($n=3$)	300 ($n=3$)
$AUC_{0-\infty}$ (pg/ml)h		3540 ± 1850	17 600 ± 3500	326 000 ± 43 000		8630 ± 670	22 400 ± 8400	265 000 ± 227 000
$AUC_{0-24\,h}$ (pg/ml)h					123	4280	16 900	91 600
C_{max} (pg/ml)	563 ± 120	19 700 ± 6300	55 700 ± 7000	787 000 ± 86 400	137 ± 7	463 ± 56	1170 ± 524	5590 ± 3413
T_{max}	0.25	0.25	0.25	0.25	5	4.33	6.67	6.67
$T_{1/2}$		1.7 ± 0.3	2 ± 0.7	2.8 ± 0.2		13 ± 5	9.3 ± 1.6	45 ± 53

Results show the mean plasma concentration ± standard deviation of 3 subjects.
AUC, area under the curve; C_{max}, maximum concentration; T_{max}, time of peak concentration; $T_{1/2}$, half-life.

of 700 pg/ml at 4 h, 603 pg/ml at 12 h and 780 pg/ml at 36 h (Table 3). Levels subsequently fell to a mean of 230 pg/ml at 48 h and were undetectable by 1 week.

DISCUSSION

This is the first report of a phase I study evaluating BB-10010, a stable variant of human MIP-1α. The primary objectives of the study were to address the safety and tolerability of BB-10010 and define the pharmacokinetics following s.c and i.v. bolus and following a 24 h s.c. infusion in subjects with normal haemopoiesis. Bone marrow examinations were not carried out as we did not expect BB-10010 to induce measurable cell-cycle inhibitory effects on normal, unperturbed bone marrow progenitor cells. Therefore, no attempt was made to evaluate the antiproliferative properties of BB-10010 in these studies.

The studies showed that BB-10010 is extremely well tolerated, thus confirming our previous experience in preclinical models and consistent with the safety profile determined by detailed toxicology studies in animals. Whilst several subjects had low levels of anti-MIP-1α antibodies at screening, no fluctuation was observed throughout the study period. Neither bolus administration (i.v. or s.c.) nor the short-lasting 24 h infusion were associated with any significant toxicity and, as anticipated, a MTD was not defined in these studies. Of particular note, BB-10010 did not invoke a local or systemic inflammatory response, contrary to previous experimental findings [1, 15] and the classification of MIP-1α as a pro-inflammatory chemokine. The lack of any pro-inflammatory effects in these studies may relate to the specific properties of BB-10010 in solution, although this would seem unlikely given the comparable biological activity of BB-10010 and native MIP-1α in other experimental systems [10]. A further possibility is that MIP-1α's pro-inflammatory effects may represent an experimental phenomenon: using a rat model, the MIP-1 doublet (α/β) was found to be pyrogenic following direct injection of protein into the region of the hypothalamus [15]. However, subsequent separation into its constituent parts showed that MIP-1β and not MIP-1α is the predominant partner during fever induction [16]. A third possibility is that MIP-1α has species-specific effects. Injection of human MIP-1α into the footpad of mice was shown to produce an immediate inflammatory response [1], whilst, in contrast, intradermal injection of hMIP-1α in dogs had no effect [17]. In the murine experiments, the MIP-1α-induced inflammatory response was accompanied by extensive mast cell degranulation and, therefore, the differing effect amongst species may, at least in part, relate to the contrasting effects of MIP-1α on mast cells between different animals [1] and, furthermore, may also explain the lack of any allergic or anaphylactic reaction in the clinical studies, despite the injection of up to 20 mg of protein.

Table 3. BB-10010 plasma levels in cancer patients following a 24 h subcutaneous infusion of 100 μg/kg

Time (h)	BB-10010 plasma levels (pg/ml)	Subject number
Pre-dose	0	3
0.5	0	3
1	49 ± 49	3
2	63 ± 63	3
4	700 ± 500	3
8	392 ± 59	3
12	603 ± 54	3
24	851 ± 152	3
36	780 ± 110	3
48	230 ± 28	3

Results show the mean plasma concentration ± standard error of 3 subjects.

In contrast to the findings in mice [6], BB-10010 had no significant enhancing effect on the total leucocyte count or on neutrophil numbers in these trials. Although hMIP-1α may be chemotactic for neutrophils in mice [1, 18], the evidence for such an effect in humans is in fact weak. McColl and colleagues [19] reported that hMIP-1α exerted a small dose-dependent increase in intracellular calcium in human neutrophils but this was minor in comparison with the increase associated with the C–X–C chemokines, IL-8 and GROα. Furthermore, the calcium mobilising effect following MIP-1α was not coupled to neutrophil effector functions such as degranulation or chemotaxis.

Administration of BB-10010 did result in a dose-related, relative monocytosis, but this did not reach statistical significance. This effect appears to be specific, not only in terms of the dose relationship, but also in view of the fact that the response mirrored the BB-10010 pharmacokinetics. This finding is consistent with the experimental evidence that MIP-1α and related C–C chemokines act principally as chemotactic factors for mononuclear cells [19, 20]. The increase in monocytes was most apparent following s.c. injection and was frequently preceded by an acute, short-lasting reduction in monocyte numbers, a phenomenon that has been documented following GM-CSF administration in which the neutrophil leucocytosis was preceded by an initial acute fall in neutrophils [21], possibly as a consequence of leucocyte–endothelial interactions or pooling in the pulmonary circulation.

BB-10010 did not appear to influence the release of bone marrow progenitor cells significantly. In mice, BB-10010 resulted in a maximal 2-fold increase in circulating progenitor cells when used alone [6]. In the clinical studies, a 3-fold increase in the total colony-forming cells was observed at the 10 μg/kg dose level, but this was not dose related and there was no measurable effect on the GM-CFC population. Given the wide variation in the mobilising potential between individuals and the small subject numbers used in these preliminary studies, it is perhaps not surprising such a small effect was not detected. We did consider that the lack of a clear mobilising effect may have resulted from the differing pharmacokinetic profiles of bolus BB-10010 injection in different species. Following s.c. administration of 100 μg/kg BB-10010, the peak plasma concentration observed in man was 10-fold less than that seen in mouse, rat and marmoset. Similar peak plasma levels of BB-10010 to those obtained with 100 μg/kg in a mouse were achieved in humans following 300 μg/kg s.c. or 30 μg/kg i.v., but neither dose level had any additional effect upon mature cell numbers and progenitor cell mobilisation following 30 μg/kg i.v. was similar to that observed following dosing with 100 μg/kg s.c.

Pharmacokinetic analyses showed that BB-10010 plasma levels were detectable despite an inability to determine any clinically significant effect on mature and progenitor cell levels. When administered as a single s.c. injection of 30–300 μg/kg, BB-10010 produced a sustained plasma concentration over a 24 h period, suggesting that a once daily injection is adequate for further clinical evaluation. Subcutaneous infusion represents an alternative method of administration and is a particularly attractive mode of delivery for investigating the effects of protracted exposure to BB-10010 and may overcome any potential difficulties associated with high dose administration. Sustained plasma levels of BB-10010 were achieved over a 36 h period, but C_{max} values were significantly less than those achieved with a corresponding s.c. bolus. There was a trend towards higher mean concentrations of BB-10010 in the advanced cancer patient study as compared with the corresponding s.c. dose in the volunteers, these results presumably reflecting different subject populations (female cancer patients versus healthy male subjects) with differing fat distribution and perhaps differing drug clearance.

In conclusion, these initial phase I studies have shown that BB-10010 is extremely well tolerated with no significant toxicity up to 300 μg/kg. A daily s.c. injection of ≥ 30 μg/kg resulted in measurable plasma levels and a dose-related monocytosis. Plasma levels achieved using the 300 μg/kg dose equated to those required for *in vivo* activity. Although a significant biological effect was not detected, this was neither a primary objective of the studies nor should any be expected in a short-term study of individuals with normal haemopoiesis. After confirming the safety of BB-10010 up to 300 μg/kg, we have now embarked upon further clinical studies to investigate the activity of a variable dose and duration of BB-10010 using the above dose range in combination with cytotoxic chemotherapy. Further dose escalation studies of BB-10010 clearly need to be considered, but observations on other regulators of haemopoiesis, albeit growth factors, do suggest that these molecules may be active at much lower doses than those required for *in vivo* activity in rodent systems, e.g. G-CSF.

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