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Original article

Safety and patient response as indicated by biomarker changes to binding immunoglobulin protein in the phase I/IIA RAGULA clinical trial in rheumatoid arthritis

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Abstract

Objectives. Binding immunoglobulin protein (BiP) is a human endoplasmic reticulum-resident stress protein. In pre-clinical studies it has anti-inflammatory properties due to the induction of regulatory cells. This randomized placebo-controlled, dose ascending double blind phase I/IIA trial of BiP in patients with active RA, who had failed accepted therapies, had the primary objective of safety. Potential efficacy was measured by DAS28-ESR and changes in biomarkers.

Methods. Twenty-four patients with active RA who had failed one or more DMARDs were sequentially assigned to three groups each of eight patients randomly allocated to receive placebo (two patients) or BiP (six patients), 1, 5 or 15 mg. Patients received a single i.v. infusion over 1 h and were observed as inpatients overnight. A 12-week follow-up for clinical, rheumatological and laboratory assessments for safety, efficacy (DAS28-ESR) and biomarker analysis was performed.

Results. No infusion reactions or serious adverse drug reactions were noted. Adverse events were evenly distributed between placebo and BiP groups with no BiP-related toxicities. Haematological, renal and metabolic parameters showed no drug-related toxicities. Remission was only achieved by patients in the 5 and 15 mg groups, and not patients who received placebo or 1 mg BiP. Good DAS28-ESR responses were achieved in all treatment groups. The BiP responding patients showed significantly lower serum concentrations of CRP, 2 weeks post-infusion compared with pre-infusion levels, and of VEGF and IL-8 from the placebo group.

Conclusion. BiP ($\leqslant$15 mg) is safe in patients with active RA. Some patients had clinical and biological improvements in RA activity. BiP merits further study.

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Key words: first-in-human trial, safety, efficacy, changes in biomarkers, VEGF, IL-8, CRP, regulatory cells, prolonged activity, BiP

CLINICAL SCIENCE

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Rheumatology key messages

- Intravenous binding immunoglobulin protein is safe in this first clinical trial in patients with unresponsive RA.
- Binding immunoglobulin protein induces significant falls in the biomarkers CRP, VEGF and IL-8 in RA despite high background placebo clinical responses.
- After a single intravenous infusion, binding immunoglobulin protein may induce remission lasting up to 3 months in RA patients.

Introduction

The treatment of RA has been transformed by the use of targeted protein biologics [1]. Despite these advances in the management of RA, the great unmet need is the development of therapies that induce cure: prolonged periods of drug-free remission [2]. Anti-cytokine biologics are only functional while an effective serum concentration is maintained, that is, pharmacodynamics and pharmacokinetics (PK) are concordant requiring frequent repeat dosing [3]. In contrast, rituximab, an anti-CD20 antibody, by killing $CD20^+$ B cells, can have a prolonged beneficial clinical effect, that is, pharmacodynamics in excess of PK [4]. However, rituximab has the disadvantage [5] of being a cellular ablative therapy.

There is now overwhelming evidence that immune response homeostasis is maintained by immune regulatory cells of varying descriptions but mainly B cells [6] and T cells [7]. The induction of regulatory cells may be the best prospect for developing curative therapies for RA as defined above [8]. Developing cellular therapies is confronted by the dual problems of consistent methods for expanding regulatory cell populations and then determining optimal doses and frequency of administration [9, 10]. Stress proteins have been demonstrated to generate regulatory functions, suggesting they may be a suitable alternative way of generating this response. Our preclinical studies have shown that systemically administered binding immunoglobulin protein (BiP), also known as 78 kDa glucose regulated protein, an endoplasmic reticulum resident chaperone and stress protein, has potent anti-inflammatory and immunomodulatory properties [11]. More pertinently BiP, through deactivation of human monocytes and abrogation of dendritic cell maturation, leads to induction of CTLA-4^+ regulatory T cells [12]. A single injection of BiP in mice with collagen-induced arthritis delivers a prolonged, sustained therapeutic response that can be transferred by BiP-sensitized spleen and lymph node cells in the absence of additional BiP [12]. Furthermore, a single i.v. dose of BiP in mice with severe combined immunodeficiency, bearing s.c. transplants of RA synovial membrane, led to suppression of rheumatoid inflammation [13]. This model has been used to test biologics, such as anti-TNFα and anti-IL-6 receptor antibodies [14, 15].

We hypothesize that an i.v. infusion of BiP should have a prolonged therapeutic effect. The Rheumatoid Arthritis Regulatory (RAGULA) trial is a Phase I/IIA randomized placebo-controlled, dose escalating, first-in-man clinical trial, designed firstly to test for safety; secondly, to test for efficacy in patients with active RA who have failed DMARDs and, in some patients, biologics; and, thirdly, to explore PK and biomarker data as related to clinical and biological endpoints following infusion of BiP.

Methods

Study design

This study was a double-blind, randomized, placebo-controlled, parallel group, single ascending dose design in three treatment cohorts of eight female or male patients with active RA as stipulated by the Medicines and Healthcare Products Regulatory Agency (MHRA). Within each treatment cohort, six patients were randomized to receive BiP and two to receive placebo (normal saline). Three doses of BiP, 1.0, 5.0 and 15.0 mg, were investigated in ascending order. The study was approved by the MHRA (reference 40945/0001/001-0001) and the London Bridge Research Ethics Committee (12/LO/0012). It was performed in accordance with the principles of the Declaration of Helsinki. Signed informed consent for the study was obtained from each patient before any study-related procedures were undertaken. The full study protocol is available from the sponsor. An independent Data Monitoring and Safety Committee based outside our institutions reviewed all clinical and laboratory results for safety after each dosing cohort and gave consent to proceed to the next dosing level.

Following screening, eligible patients were admitted within 1 week to the Quintiles Drug Research Unit. Patients were admitted on day 1 for final eligibility checks and baseline assessments, and then randomized to receive BiP or placebo. The randomization list was generated by the study statistician using the R statistical package and maintained on the MedSciNet database for authorized access as patients passed final safety tests at the Quintiles facility. A sentinel strategy was employed, where, of the first two patients in each cohort, one was randomized to receive BiP, and the other placebo. On the morning following admission, patients received a single i.v. infusion of BiP or placebo over 1 h. There was an appropriate stagger of at least 24 h between dosing each patient in each cohort. Patients were monitored for 24 h following infusion and discharged from the Quintiles Unit on day 2 after final assessments had been performed. Following discharge, patients were assessed for safety and efficacy at weeks 1, 2, 3, 4, 8 and 12, at the Guy's Hospital Clinical Research Facility.

Patients

The study randomized 24 patients aged from 18 to 75, with RA as defined by the 1987-revised ACR diagnostic criteria [16] for at least 6 months with active RA defined by

having at least six swollen and six tender joints, CRP >4 mg/l and/or ESR >15 mm/h despite adequate dosage of at least one DMARD with a normal chest X-ray within 3 months of randomization. Major exclusion criteria were treatment with any biologic drug within 3 months of screening (6 months for rituximab), functional Class IV by ACR Criteria [17], safety screening pathology results outside pre-defined ranges, hepatitis B or C, HIV positive, and any other active systemic infection within 2 weeks before baseline. Patients with a history of malignancy (except basal cell carcinoma or adequately treated carcinoma *in situ* of the cervix), significant cardiac, renal, neurological, psychiatric, endocrine, metabolic or hepatic disease were also excluded. Patients could continue the following medications at a stable dose for at least 4 weeks before the baseline visit and during the study: SSZ (up to 3 g/day), MTX (up to 25 mg/week), HCQ (up to 400 mg/day), LEF (up to 20 mg/day), prednisolone or prednisolone equivalent (up to 10 mg/day) and NSAIDs.

Doses of BiP

BiP (recombinant human BiP) was produced under good manufacturing practice guidelines by the NHS Blood Transfusion Clinical Biotechnology Centre, University of Bristol, Bristol, UK, stored as frozen liquid (5 mg/ml dose vials) at $-80^{?}$C. As this was the first-in-human dosing of BiP, the MHRA requested the first dose should be just within the therapeutic range as determined in pre-clinical animal studies. Pre-clinical models showed no toxicity over a wide dose range, so a no observed adverse effect level dose was not achieved to calculate a possible highest dose. Based on pre-clinical modelling three doses were chosen, 1 mg/patient (cohort 1), 5 mg/patient (cohort 2) and 15 mg/patient (cohort 3).

Study endpoints

Safety

The primary end point was safety, assessed clinically, and by laboratory and ECG measures. During the inpatient admission at Quintiles clinical trials unit, ECG was performed twice prior to infusion, then hourly for 4 h, then twice again before discharge. Changes in laboratory safety measures were graded using the Common Terminology Criteria for Adverse Events [18].

Efficacy

The main efficacy end point was DAS28-ESR response, graded according to the EULAR response criteria [19] into good, moderate and non-response with remission defined as a DAS28-ESR <2.6 and the ACR 20, 50 and 70 responses [20]. Biological efficacy endpoints were changes in ESR and CRP.

Exploratory PK and biomarkers

PK. Serum BiP concentrations were measured by a sensitive ELISA technique developed in our laboratory at the screening visit and at 24 h after the i.v. infusion of BiP. These values also include endogenous BiP as the two molecules could not be distinguished.

Serum VEGF and IL-8 concentration. Gene array data (Valerie M. Corrigall and Gabriel S. Panayi, unpublished data) showed that VEGF and IL-8 production from human peripheral blood monocytes was inhibited by BiP. Serum VEGF and IL-8 concentration was measured before infusion and at 2 and 12 weeks by Luminex technology (Bio-Rad, Hemel Hempstead, UK). Only patients remaining in the study at 12 weeks were included in this analysis.

Statistical analysis

Safety

Due to the exploratory nature of this study, no formal sample size calculations were performed. The study design was based on the results of the pre-clinical studies. With six subjects per cohort receiving BiP, the probability of observing at least one patient with an adverse event is $\geqslant 90\%$, for an underlying event rate $\geqslant 33\%$. A cohort of size 8, with no observed events in the six active patients, would provide a 95% CI of 0–46% for the underlying adverse event rate.

Efficacy

It was expected that the last two doses would show significant benefit. For descriptive statistics, mean and S.E. were used for continuous secondary outcome, namely DAS28 score. Plots with these summary statistics as a function of time were performed to show how these measurements change over time. The AUC over time for each participant was used as a summary accumulated effect and described for each drug group using means and 95% CIs. Differences between placebo and the three dose groups were tested using two-sample t-tests or Mann–Whitney tests, and non-randomized comparisons of the changes within cohorts involved paired t-tests and Wilcoxon tests. For response thresholds, the proportion of patients achieving the corresponding response at each time point for each drug group were described. Effects were considered significant if $P < 0.05$.

Biomarkers

Biomarker data were analysed using non-parametric tests, non-paired, Mann–Whitney and paired Spearman rank tests where appropriate.

Results

Forty-two patients were screened, and 24 were randomized to receive either BiP or placebo. The demographics of the patients are shown in Table 1. The four groups of patients, placebo and cohorts 1, 2 and 3, were comparable in most demographic data (Table 1), with the exception that no male received placebo, and the groups who received 1 and 5 mg doses had failed more therapies. Most patients had failed multiple DMARD therapies, and remained on a DMARD during the study. Four patients had failed up to eight biologic therapies, three in cohort 1 and the fourth in cohort 2. Three patients were not on current DMARD therapy. Most patients completed the study after infusion. In the placebo group, no patient

Table 1 Demographic details of patients recruited into the RAGULA trial

Characteristics	BiP 1 mg (n = 6)	BiP 5 mg (n = 6)	BiP 15 mg (n = 6)	Placebo (n = 6)	Total (n = 24)
Female/male, n (%)	6/0 (100/0)	6/0 (100/0)	3/3 (50/50)	6/0 (100/0)	21/3 (87/13)
Age, mean (S.D.), years	50.8 (7.6)	53.5 (10.4)	53.5 (11.7)	56 (10)	52.5 (9.5)
Race, Caucasian, n (%)	5 (83)	6 (100)	4 (67)	4 (67)	19 (79)
Disease duration, mean (S.D.), years	11.3 (4.3)	12.3 (9.3)	11.5 (11.6)	8 .2	10.5 (10)
RF positive, n (%)	5 (83)	6 (100)	5 (83)	3 (50)	19 (79)
DMARD concurrent use, n (%)	4 (66)	5 (83)	5 (83)	5 (83)	19 (79)
MTX	1 (17)	4 (66)	4 (66)	5 (83)	14 (58)
HCQ	0	1 (17)	1 (17)	1 (17)	3 (13)
SSZ	2 (33)	5 (87)	1 (17)	1 (17)	9 (37)
LEF	0	0	0	1 (17)	1 (4)
Prednisolone	3 (50)	1 (17)	1 (17)	1 (17)	6 (25)
Failed biologics, n (%)	3 (50)	1 (17)	0	0	4 (17)
Infliximab	1 (17)	1 (17)	0	0	2 (8)
Adalimimab	3 (50)	0	0	0	3 (13)
Etanercept	2 (33)	1 (17)	0	0	3 (13)
Certolizumab	1 (17)	0	0	0	1 (4)
Golimumab	1 (17)	0	0	0	1 (4)
Rituximab	2 (33)	0	0	0	2 (8)
Tocilizumab	2 (33)	0	0	0	2 (8)
Abatacept	2 (33)	0	0	0	2 (8)

BiP: binding immunoglobulin protein; RAGULA: Rheumatoid Arthritis Regulatory.

was withdrawn because of worsening disease; in cohort 1, two patients were withdrawn at week 4 for worsening disease and another patient withdrew at week 8, because she wished to travel overseas while she felt so well. In cohort 2, one patient was withdrawn at week 4 because of active disease, and in cohort 3 one patient was withdrawn at week 4 for active disease and one patient at week 8 because of a protocol violation: she discontinued methotrexate therapy as she felt so well.

Primary endpoint: safety

No serious adverse events were observed at the time of the infusion of BiP, during the 24 h inpatient observation period or the subsequent 12-week follow-up. No patient was withdrawn because of adverse events, and no pattern of adverse events was noted that could be ascribed to BiP. Adverse events occurred in 23 subjects, evenly distributed among all groups, and none required specific intervention (Table 2). Laboratory safety measures also did not change in a way that suggested any drug-related toxicity, with changes occurring in all groups including those who received placebo. All changes were Common Terminology Criteria for Adverse Events Grade I, with the exception of one subject in the 15 mg group with grade II haemoglobin values at pre-treatment and visit 4. In supplementary Tables S1 and S2, available at *Rheumatology* Online, complete laboratory safety monitoring values are given. ECGs showed no medically relevant changes throughout the monitoring period.

Secondary endpoint: clinical efficacy

In terms of the EULAR response criteria (Table 3) at 12 weeks, there was one good, four moderate and one non-responder in the placebo group; one good, one moderate and four non-responders in cohort 1; two good, one moderate and three non-responders in cohort 2; and two good, one moderate and three non-responders in cohort 3. DAS28 area under the curve analysis over the 12-week period did not contribute any more useful data. At 12 weeks, remission was achieved by one patient (17%) in cohort 2 and by two patients (33%) in cohort 3, and no placebo patient or patient in cohort 1. In those patients who achieved remission, low DAS28 scores were seen by 3 weeks (2.77, 2.61 and 2.72) (Table 3).

Sustained ACR20 responses (defined as ACR20 response in at least half of all follow-up visits) were seen in 33% of placebo, cohorts 1 and 2 patients and in 66% of cohort 3 patients. ACR50 and 70 responses were seen in subjects who achieved good EULAR responses.

On analysis of the change from pre-infusion levels in patients' serum CRP levels in the placebo group, a significant increase ($P = 0.015$) was observed over 12 weeks (Fig. 1). In the responders to BiP treatment there was a strong trend to reduced CRP at 2 weeks, although not significant [2 weeks: placebo, -1.2 (2.7) *vs* BiP responders, -5.6 (5.3); $P = 0.09$], although at 12 weeks the change in serum CRP levels was significantly lower in the total BiP-treated group than the placebo group [12 weeks: placebo, 3.5 (3.4) *vs* total BiP-treated patients, -0.6 (10.6), $P = 0.051$] despite the inclusion of the high CRP level of the protocol violator.

Exploratory PK and biomarker detection

PK

The ELISA for the detection of administered BiP, a homologue of the endogenous protein, does not distinguish

Table 2 Clinical adverse events affected >10% of the study population

Adverse event	BiP (n = 6/group)			Placebo(n = 6)	Total(n = 24)
	1 mg	5 mg	15 mg		
Upper respiratory tract infection	1	2	4	2	9
Limb pain	1	3	3	2	9
Diarrhoea/gastroenteritis	0	1	4	3	8
Headache	2	2	1	2	7
Backache	1	1	1	2	5
Abdominal pain	0	0	3	1	4
Nausea	1	0	1	1	3
Dizziness	1	0	0	2	3

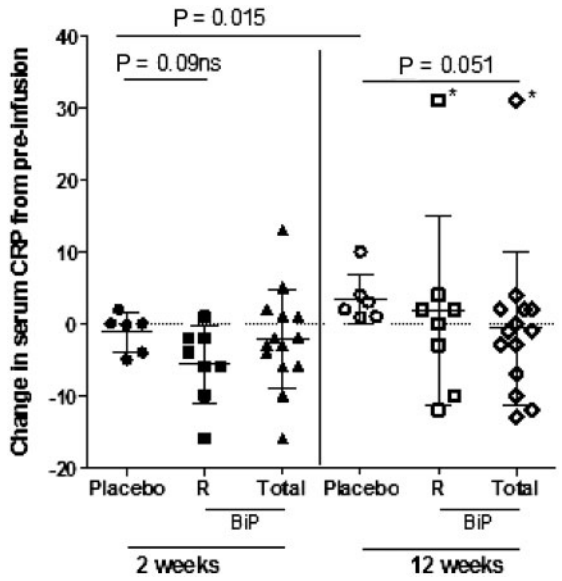
Each group shows the number of events recorded during the 54 follow-up visits and is independent of the number of patients affected by any adverse event. There was no significant difference between the groups in the prevalence, severity or type of adverse events. BiP: binding immunoglobulin protein.

Table 3 DAS28-ESR values and remission responses

Patient ID	Visits								EULAR response at W12
	0 Screen	2 W0	4 W1	5 W2	6 W3	7 W4	8 W8	9 W12	
				Placebo					
1	5.00	6.73	4.53	3.72	6.07	4.18	5.10	3.75	Moderate
3	4.90	4.96	4.62	3.66	3.68	3.99	3.93	3.48	Moderate
9	6.43	6.49	5.51	5.40	5.21	5.74	6.44	6.33	None
14	5.44	5.45	3.99	4.19	3.70	4.47	3.69	4.63	Moderate
18	6.68	6.92	6.10	6.17	6.08	4.94	5.87	4.84	Moderate
20	4.66	5.89	1.71	**3.37**	4.30	3.73	**2.67**	**2.63**	Good
				1.0 mg BiP					
2	5.17	5.69	5.10	5.07	5.85	4.08	5.98	6.43	None
4	6.35	6.02	6.42	5.92	4.59	5.12	6.00	5.45	None
5	8.13	8.28	7.72	8.24	8.37	8.56	8.56	8.56	None
6	6.77	6.52	6.08	6.93	7.00	7.62	7.62	7.62	None
7	7.49	7.49	4.43	4.18	3.40	3.24	3.58	3.04	Good
8	6.38	6.43	5.72	5.99	5.47	5.14	4.95	4.95	Moderate
				5.0 mg BiP					
10	5.03	5.41	4.83	5.82	5.78	6.03	5.13	4.85	None
11	7.35	7.30	7.85	7.49	7.12	7.51	7.72	7.72	None
12	4.18	2.95	4.04	**2.19**	2.93	3.36	3.14	2.92	Good
13	4.45	4.53	4.24	2.97	2.94	4.03	3.69	3.22	Moderate
15	4.28	4.15	3.12	**2.66**	2.77	3.46	2.77	**2.47**	**Good/remission**
16	5.59	6.42	5.59	5.02	5.07	4.96	5.50	6.39	None
				15.0 mg BiP					
17	7.04	6.34	6.81	6.57	6.9	5.87	6.48	6.16	None
19	4.62	4.52	3.52	3.22	3.48	3.58	4.49	4.94	None
21	5.78	6.07	6.63	5.87	6.40	6.66	6.66	6.66	None
22	4.72	4.38	3.41	3.35	**2.61**	3.06	3.40	**2.33**	**Good/remission**
23	4.37	4.56	4.26	3.60	2.72	3.06	**2.52**	**2.52**	**Good/remission**
24	6.89	6.84	7.20	7.02	5.72	5.81	5.62	5.62	Moderate

DAS28-ESR shown in bold are patients in remission at that visit. Patient ID: patient identity number; Screen: screening visit; W: week.

Fig. 1 Serum CRP levels



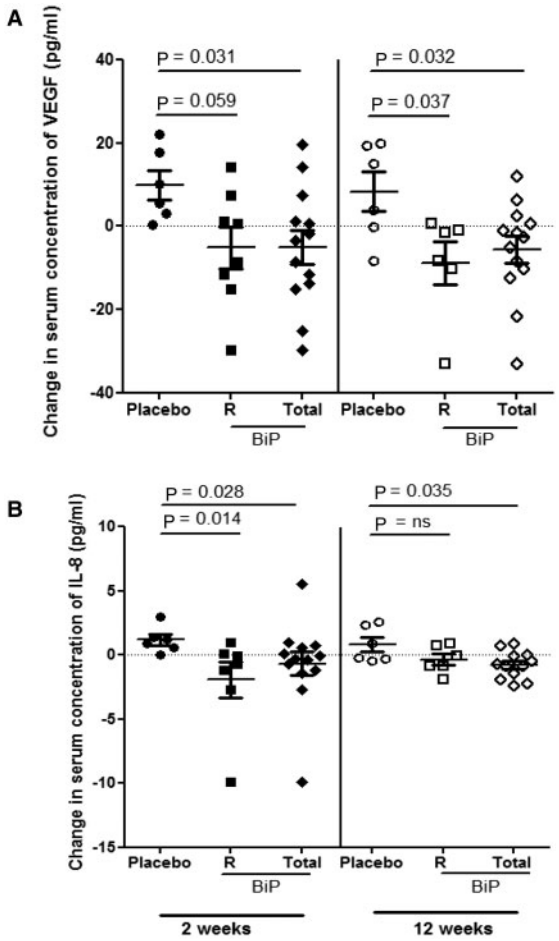
CRP was quantified in serum from patients taken pre-infusion, and at 2 and 12 weeks post-infusion. Three groups of patients are shown, placebo, BiP responders (R) and BiP non-responders (NR). Asterisks denote protocol violator who had ceased concurrent medication.

between the proteins. Individual serum levels of BiP in all groups of patients, whether placebo or those receiving the drug, did not change from baseline over the initial 24 h period after the infusion.

Biomarker detection: serum VEGF and IL-8 concentrations

Due to the small numbers in each treatment group, biomarker analysis was undertaken according to whether the patient showed a EULAR response or not (Table 3). The six placebo patients were kept as a group regardless of their EULAR clinical response. Both at 2 and 12 weeks post-infusion, when compared with those patients receiving placebo, serum VEGF levels in BiP-treated patients were significantly reduced. Unlike the BiP responder (R) patient group or the total group of BiP-treated patients, placebo patients generally showed increased serum levels [change from pre-infusion at 2 weeks: placebo, 9.9 (8.5) pg/ml, range, 0.5–22.0 pg/ml *vs* BiP responders, −7.9 (12.5) pg/ml, range, −29.9 to 7.5 pg/ml, P = 0.059; or total treated group, −5.06 (14.4) pg/ml, range, −29 to 19.8 pg/ml, P = 0.031. Twelve weeks post-infusion: placebo, 8.3 (11.6) pg/ml, range, −8.4 to 19.9 pg/ml *vs* BiP responders, −8.9 (12.1) pg/ml, range, −33.0 to 2.6 pg/ml, P = 0.028; or total treated group, −5.7 (11.9), range, −33 to 12.2 pg/ml, P = 0.032] (Fig. 2A). Similarly, changes in serum IL-8 were significantly lower at 2 weeks but less so at 12 weeks post-infusion (Fig. 2B) when compared with the placebo group [2 weeks: placebo,

Fig. 2 Changes in biomarker levels in BiP-treated patients



Serum concentrations of VEGF and IL-8 were measured by Luminex bead technology and the change from pre-infusion serum concentration calculated for each patient at 2 and 12 weeks. (**A**) Change in VEGF concentration; (**B**) change in IL-8 concentration. Data show placebo group (n = 6), responder group (R) [n = 8 (2 weeks) and 6 (12 weeks)] and the total patient group treated with BiP [n = 14 (2 weeks) and 12 (12 weeks)] who remained in the study at 12 weeks. Range of concentration (all patients) VEGF, 4–195 pg/ml; and for IL-8, 0.7–19 pg/ml.

1.2 (1) pg/ml, range 1–3 pg/ml *vs* BiP R, −0.6 (1.2) pg/ml, range −2.7 to 1 pg/ml, P = 0.014; or total treated group, 0.09 (2) pg/ml, range −2.7 to 5.5 pg/ml, P = 0.028; 12 weeks post-infusion: placebo 0.8–1.4 pg/ml, range −0.5 to 2.6 pg/ml *vs* BiP R, −0.37 (1.1), range −1.9 to 0.9 pg/ml, P = NS; or total treated group, −0.8 (1.1) pg/ml, range, −2.4 to 0.9 pg/ml, P = 0.035]. Within this very small number of patients no correlation between serum VEGF concentrations and CRP was observed (data not shown).

Discussion

In this exploratory clinical trial, a single i.v. infusion of BiP at increasing doses to 15 mg is safe. No BiP-related serious adverse or adverse events were observed in the clinical, laboratory tests or ECG measures.

Efficacy was the secondary outcome, with biological markers being increasingly used to detect efficacy in small studies [21]. A very heterogeneous group of patients was entered into this safety study, compared with those entering large efficacy studies. Consequently, this trial was confounded by a high level of clinical response in the placebo group, which makes judgement of efficacy difficult. Clinically, good EULAR responses were more common in those treated with higher doses of BiP with sustained low DAS28 scores (from 3 to 12 weeks) observed in three patients who received BiP, compared with no patients who received placebo. These findings parallel our pre-clinical observations in the murine CIA model in which the response occurred early and was sustained for several weeks after a single dose of BiP [12]. As in many early Phase II studies with variable patient characteristics, analysis of biomarkers proved useful in differentiating subjects receiving active drug compared with placebo. Patients who responded to BiP showed a significant decrease in CRP at 2 weeks, compared with the placebo and non-responder groups. Serum VEGF and IL-8 are common biomarkers used in biologic clinical trials because they correlate well with measurement of synovitis [22, 23] and monocyte infiltration [24, 25], respectively. Significant changes in levels of these biomarkers occurred in patient groups receiving BiP. Furthermore, biomarkers did not support clinical improvement in placebo patients. Strikingly, at week 12 significantly fewer patients who received placebo showed reduced serum VEGF and IL-8 (17 and 50%, respectively), compared with the BiP responder group (71 and 83% of patients, respectively). Interestingly even the BiP non-responder group showed reduced serum concentrations (66 and 83% of patients, respectively), suggesting a change in the pathology of their disease. Further studies are needed to confirm these data and to establish optimum dose and frequency of administration of BiP.

The Rheumatoid Arthritis Regulatory trial should be seen in the context of a long history of research suggesting that microbial and human stress proteins, HSP and their specific peptides can induce regulatory T cell differentiation and down-regulate inflammatory responses. These consistent pre-clinical findings triggered clinical trials of the proteins or their peptides as novel therapies for type 1 diabetes (HSP60 DiaPep277 [26]), JIA (HSP dnaJ-derived epitope) [27] and RA [28]. These early phase clinical trials were inconclusive, partly due to sub-optimal study design, for example, the trial of the HSP chaperonin 10 in RA [29] had no placebo arm, making interpretation of effects difficult. Thus the present findings with BiP are exciting and novel. To our knowledge this is the only double-blind, placebo-controlled, fully randomized clinical trial of a human stress protein in an autoimmune disease that shows safety and suggests efficacy. The prolonged therapeutic activity suggests induction of regulatory cells, supporting the original hypotheses regarding therapeutic action of stress proteins. Further biomarker analysis is underway to provide definitive proof of concept.

In summary, a single i.v. infusion of BiP ($\leqslant$15 mg) is well tolerated with no drug-related adverse events. There are suggestions of clinical efficacy supported by biological end points. Larger studies are required to confirm and expand these data, particularly the optimum dose and frequency of BiP administration. Immunological analysis of cellular and humoral biomarkers monitored during the trial should reveal whether BiP can induce regulatory cell activity. In that event BiP may have therapeutic uses in other indications such as JIA, spondylitic diseases and PsA.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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