PHASE I STUDY OF STAPHYLOCOCCAL PROTEIN A IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS ON METHOTREXATE

Eular Paris 2014

Craig Wiesenhutter [1] Alan Kivitz [2] Jeffrey Kaine [3] John Lavery [4] Lydie Hazan [5] Edward Bernton [6]

[1]Univ. Washington School of Family Medicine, Seattle and Coeur D'Alene Arthritis Center, Coeur D'Alene, [2]Altoona Clinical Research Center, Altoona, [3]Sarasota Arthritis Center, Sarasota, [4]Allen Arthritis and Allergy, Allen, [5]Axis Clinical Trials, Los Angeles, [6]Protalex, Inc, Floham Park

CONCLUSIONS

- Weekly infusions of SpA were well-tolerated in patients with active RA, with most common side-effects being a transient flare of RA joint inflammation and fatigue.
- The plasma Cmax showed a linear relationship with dose, but clearance and AUC changed with repeated doses in patients who developed anti-SpA antibodies.
- About 30% of active-treated patients and 12% of placebo-treated patients had an ACR50 response by 4 weeks after the last SpA infusion. This response appeared correlated with RA flares during treatment. Some apparent responders included patients who developed high-titer anti-SpA Abs..

Introduction

Staphylococcal protein A (SpA) is a bacterial virulence factor which structurally consists of five homologous immunoglobulin (Ig) binding domains placed in tandem (1). Each SpA domain is capable of binding with high affinity to the Fc region of most human Igs, which has led to its wide use in chromatography columns used to purify IgG products. But SpA also binds to the Fab framework region of immunoglobulins in the mammalian V_H3 gene family, with equal or higher affinity, and 30-40% of human B cells utilize the Vh3 heavy chain. This ability to bind to Vh3 immunoglobulins and to Vh3 B-cell receptors result in SpA one of the best characterized mammalian B-cell superantigens. Another is the HIV gene product, GP120.

Small doses of parenterally-administered SpA, in the presence of large excesses of serum IgG, form complexes consisting of 2 molecules of SpA and 4 molecules of IgG (2). These molecules may function as “immune-complex mimetics” and induce “alternatively-activated” or “regulatory” macrophages, characterized by an anti-inflammatory phenotype, down-regulated MHC-II, decreased TNF-alpha secretion after LPS stimulation, and increased IL-10 secretion (3).

The No Observable Adverse Effect level for weekly injections of SpA in cynomolgus monkeys is >100 µg/kg. In patients with active rheumatoid arthritis on methotrexate, SpA was safe and well-tolerated in a phase I trial when given as 4 weekly i.v. injections at doses up to 1.5 µg /kg. That trial suggested that in some patients, 4 doses of SpA could induce delayed, but sustained decreases in RA activity. The current study was conducted to evaluate safety, pharmacokinetics, immunogenicity, and effect on RA disease activity of 5 weekly doses of 1.5, 3.0, 6.0, or 12 µg/kg of intravenous SpA, and to examine exploratory endpoints such as changes in circulating lymphocyte phenotypes and the generation of complement activation products after SpA dosing.

Methods

Patients with active RA (≥4 swollen and ≥ 5 tender, DAS 28 joint count) on stable therapy with either methotrexate or leflunamide were randomized. At least 6 months of disease duration and either a positive RF or anti-CCP titer were required. Patients on > 10 mg/day prednisone equivalent were excluded, and prednisone doses were kept constant during the study period. Sequential cohorts were dosed at a 3:1 ration with increasing doses of active drug or placebo (doses of 1.5, 3.0, 6.0, or 12 µg/kg, i.v.). 1.5 µg/kg doses were administered over 90 to 120 seconds. 3.0 and 6.0 µg/doses were infused over 30 minutes, and 12 µg/kg doses were infused over 1 hour.

After 5 weekly infusions with SpA drug product (Days 1 through 29), safety and disease activity were evaluated at days 57, 85, 113, and 169. Patients with a CDAI (Clinical Disease Activity Index) of > 14 at Day 85 could elect to exit the study per protocol to pursue alternative treatments. Triplicate EKG’s were performed before and 30-60 minutes after dosing on Days 1 and 29 and evaluated for morphology, rhythm, and change in QTc intervals. Complete plasma PK profiles (through 72 hrs post-dose) were obtained during and after infusions on Days 1 and 22 for PK parameter derivation. CBC’s were evaluated at days 1, 2, 15, 22,23, 29, 57, and 169 or EOS visit. Urinalysis and blood chemistry were evaluated at Days 1, 15, 29, 57, and 169 or EOS visit. Lymphocyte phenotyping for CD19+, CD3+/CD4+, CD3+/CD8+, CD16+/CD56+, and CD19+/Vh3+ was performed at Days 1, 22, 23, and 57. Before and after the first and 4th weekly dosing, futhan-stabilized plasma was frozen for analysis of stable metabolites of complement components C3a, C4a, and C5a using a sensitive cytometric bead array assay (Becton Dickenson, Mountain View, Ca). Anti-SPA antibodies were measured with a validated ELISA on Days 1, 22, 57, and 169. For disease activity assessments, the primary endpoint was the ACR 20/50/70 response at day 85 or the categorical CDAI response (#/percent with CDAI ≤10 or ≤ 22) at day 85. Thirty-seven of the 41 randomized patients completed the study per protocol. Twenty-nine subjects completed the day 169 visit, while 4 subjects exited per protocol at sponsor’s discretion after their day 85 or day 113 visits. Per protocol analysis population was defined as patients who completed at least 4 of 5 dosing visits and had no major protocol violations. Four (12.9%) of active-treated and 4 (40%) of placebo patients discontinued before completing Day 169 of the protocol.

Patient Demographics (per protocol patients)

	SpA 1.5 µg/kg	SpA 3.0 µg/kg	SpA 6.0 µg/kg	SpA 12 µg/kg	ALL SpA	PLACEBO
# of subjects	6	9	9	5	29	8
Mean Age	61.2 (13.3)	57.6 (11.8)	63.3 (9.7)	62.6 (6.8)	61.1 (10.3)	59.9 (9.4)
Age Range	47 – 76	30 – 72	42 – 71	51 – 72	30 – 72	46 - 71
Male	66.7%	44.4%	33.3%	57.1%	48.4%	30%
Caucasian	83.3%	88.9%	77.8%	85.7%	83.9%	80.0%
Weight (kg)	74.6 (4.3)	91.4 (20.6)	81.0 (16.2)	86.0 (16.9)	83.9 (16.9)	82.3 (14.0)
Day 1 DAS28-CRP	4.9 (0.55)	5.3 (0.62)	4.8 (0.67)	4.65 (0.88)	4.93 (0.68)	5.30 (0.87)
Day 1 DAS28 Swollen Joints	10.2 (4.54)	13.0 (6.4)	8.9 (4.23)	13.6 (5.18)	11.2 (5.32)	12.8 (5.50)
Day 1 CDAI	37.9 (10.7)	44.7 (12.4)	34.7 (11.3)	37.6 (14.0)	39.0 (12.1)	43.6 (13.2)
Day 8 CDAI	24.9 (12.9)	41.1 (15.7)	36.1 (15.6)	25.6 (14.7)	33.5 (15.8)	36.0 (14.8)
Day 1 CRP (range)	0.86 (1.02) 0.1, 2.6	0.29 (0.24) 0.1, 0.6	0.25 (0.18) 0.1, 0.6	0.69 (1.01) 0.1, 2.7	0.49 (0.69 0.1, 2.7	0.74 (0.66) 0.1, 2.1
RA Dx (yrs)	10.0 (2.93)	15.9 (11.7)	15.6 (11.1)	8.1 (3.9)	13.0 (9.7)	8.4 (6.8)

Safety

PROBABLY- OR POSSIBLY-RELATED TEAE (% SUBJECTS)	SpA 1.5 µg/kg (N=6)	SpA 3.0 µg/kg (N=9)	SpA 6.0 µg/kg (N=9)	SpA 12.0 µg/kg (N=7)	All SpA (N=31)	Placebo (N=10)
Subjects with one or more TEAE	***66.7***	***33.3***	***66.7***	***85.7***	***61.3***	***60***
Cardiac Disorders	0	11.1	0	0	3.2	10
Gastrointestinal Disorders	0	11.1	0	0	3.2	20
Gen Disorders(fatigue, flu-like, infus rxn)	16.7	11.1	11.1	14.3	12.9	20
Infections	0	0	11.1	14.3	6.5	0
Infusion related reactions	16.7	0	0	14.3	6.5	0
Investigations – QTc	0	11.1	0	0	3.2	0
Muscoloskeletal Disorders	**50**	**22.2**	**33.3**	**57.1**	**38.7**	**20**
Nervous system (dizziness or headache)	0	11.1	22.2	28.6	16.1	20
Respiratory (asthma)	0	0	0	0	0	10
Skin Disorders (rash)	16.7	0	0	0	3.2	0
Moderate or severe TEAE	50	11.1	22.2	71.4	38.7	30
Moderate arthralgia or fatigue	**50**	**11.1**	**22.2**	**57.1**	**32.2**	**20**

The most common treatment-related adverse events in active-treated subjects were fatigue (13%), headaches (16%), and arthritis flares (39%). There were 5 severe TEAE, 4 non-related. The related severe TEAE was dyspnea, tachypnea, chest pain occuring one hour after end of 1st infusion (12 µg/kg). No increase in plasma tryptase or anaphylatoxins occurred during this reaction. Five TEAE were serious, none of which were considered related. Moderate post-treatment joint flares and fatigue seemed more common in the 1.5 (iv push) and 12 µg/kg (1 hr infusion) treatment groups than in placebo treatment group. Infusions were given without pre-medication in most cases.

Pharmacokinetics

Dose (µg/kg) /Study Day	Infus-ion Time (min)	Dose (µg/kg) / Study Day	Cmax (ng/mL)	Cmax /dose	AUC (inf) (hr*ng/mL)	AUC (0-168) (hr*ng/mL)	T ½ (hr)	CL (mL/hr/kg)	Vz (mL/kg)
1.5 / 1	2	1.5 / 1	uu	Vv	Vv	Xx	Yy	Zz	Xx
1.5 / 22	2	1.5 / 22							
3.0 / 1	30	3.0 / 1							
3.0 / 22	30	3.0 / 22							
6.0 / 1	30	6.0 / 1							
6.0 / 22	30	6.0 / 22							
12 / 1	1	12 / 1							
12 / 22	1	12 / 22							

Displayed as mean and SD. Cmax values for 1.4 and 3.0 µg/kf were very similar, since the infusion rate was much faster for the 1.5 µg/kg group. The increase in CL and the decrease in AUC on Day 22 was entirely due to increased plasma clearance in patients who developed ADAs. However, even on Day 1, the variability in plasma clearance between patients was marked.

Incidence of Anti-SpA Antibodies (Day 22, before 4th dose) and Relationship to Increased Day 22 Plasma Clearance			
Anti-SpA Titer Day 22	***0***	***< 400***	***≥ 400***
Placebo	***7***	***1***	***0***
Active-dosed	***6***	***12***	***15***
Ratio Day 22 / Day 1 CL *	***1.77 (51.2)***	***2.95 (99.1)***	***26.5 (57.2)***
Ratio Day 22 / Day 1 AUC (0-168) *	***.70 (59.2)***	***.42 (83.0)***	***.05 (56.2)***
Ratio Day 22 / Day 1 Cmax *	***0.92 (16.7)***	***0.86 (44.3)***	***0.63 (29.7)***
# Active-dosed with CDAI ≤ 14 x 3	***4***	***3***	***5***

* Geometric Mean (Geometric % CV)

Biomarkers – Complement activation

% change in anaphylatoxin	Day 1 (post-dose / pre-dose)			Day 22 (post-dose / pre-dose)		
Mean (SD)	**C3a**	**C4a**	**C5a**	**C3a**	**C4a**	**C5a**
Placebo	-19 (13.6)	-19 (21.2)	-2 (13.7)	2 (14.7)	5 (31.2)	- 6 (23.7)
1.5 µg/kg	-5 (30.3)	9.4 (41.5)	2.7 (19.0)	54 (89.4)	-14.4 (20.1)	-13 (10.6)
3.0 µg/kg	83 (137.3)	0 (28.9)	7.3 (22.2)	266 (319)	48 (72.5)	-5 (16.4)
6.0 µg/kg	59.6 (103)	-12 (30.6)	18 (71.5)	238 (418)	-13 (48.5)	6 (27.6)
12 µg/kg	9 (14.8)	-9.8 (28.4)	-15 (37.1)	259 (166)	116 (239)	31 (51.2)

Biomarkers - For Active-treated patients, mean post-dosing C3a values were increased on day 22 vs Day 1 (225% vs 36%, P < 0.03) Active-treated patients had significantly greater C3a increases than placebo patients on both Day 1 (p =0.005) and Day 22 (p = 0.01). Increases in C3a showed no correlation with post-dosing AE’s (related) or with RA response to SpA treatment. C4a and C5a were not significantly effected by treatment. The clinical significance of 2 to 5 fold increases of C3a after the 4th dose remains uncertain, but did appear to display a dose response across increasing doses. Lymphocyte phenotyping revealed a dose-dependent transient decrease in absolute lymphocyte count at 24 hrs after Day 1 dosing for 6 and 12 µg/kg patients (-21%) vs placebo +12% or 1.5 and 3 µg/kg patients (0%) (p < 0.04) This acute PD response to SpA did not corrrelate with later disease activity responses. The only acute marker which appeared to correlate with a later treatment response was treatment-related transient RA flare. Seven of 9 (77%) patients who had treatment-related RA flares of moderate severity attained a CDAI of ≤ 10 on Day 57.

RA Disease Activity

% ACR-CRP Responders	Day 29		Day 57		Day 85		CDAI ≤ 14 on 3 consecutive visits
N = 37	ACR 20	ACR 50	ACR 20	ACR 50	ACR 20	ACR 50	
Placebo	**12.5**	**0**	**37.5**	**12.5**	**42.9**	**14.3**	**12.5**
ALL SpA	**28.6**	**13.8**	**50**	**29.6**	**48.3**	**21.4**	**31**
1.5 µg/kg	33.3	16.7	50	33.3	33.3	16.7	50
3 µg/kg	11.1	11.1	37.5	37.5	22.2	22.2	11.1
6 µg/kg	22.2	11.1	33.3	22.2	66.7	25	22
12 µg/kg	25	20	80	25	60	20	60

% CDAI ≤ 10	All sites			Site 2 excluded		
% Per Protocol Patients with CDAI ≤ 10	All sites			Site 2 excluded		
	Day 29 N = 37	Day 57 N=37	Day 85 N =37	Day 29 N = 29	Day 57 N=29	Day 85 N =29
Placebo	**12.5**	**25**	**14.3**	**0**	**12.5**	**0**
ALL SpA	**20.7**	**35.7**	**24.1**	**28.6**	**42.8**	**33.3**
1.5 and **3.0** µg/kg	**20**	**42.9**	**13.3**	**33.4**	**55.5**	**22.2**
6.0 and **12.0** µg/kg	**21.4**	**28.8**	**35.7**	**25**	**33.3**	**41.7**

Disease Activity Measures – ACR 20/50 responses appeared less sensitive to treatment effects than Clinical Disease Activity Index (CDAI) because: 1. most patients entered with a hs-CRP value < 1.0 mg/dL, and 2. unlike anti-cytokine therapies, SpA has little acute effect on CRP. A subset analysis was conducted excluding the 8 patients enrolled at site 2, as that site used different clinical evaluators across visits and had extensive variability in joint evaluations. Active-treated patients in this older population with long-established Ra seemed to be clearly dichotomized between responders (about 30% to 40% based on categorical CDAI) and non-responders. However, for both ACR and CDAI measures, more responses were seen at days 57, 85, and 113 (data not shown) for active- compared to placebo-treated patients. The peak change in CDAI occurs at day 57, 4 weeks after the last dose of study medication; this delayed onset of effect is typical of older DMARDs, but distinct from responses seen with anti-cytokine therapies. At the two higher doses, more treatment responses seemed to be maintained at Days 85 and 113 (data not shown). However the 3 µg/kg patients entered study with more severe disease. An additional cohort of 18 patients is currently completing a sub-study where the 5 weekly SpA treatments are followed by 4 monthly treatments on days 57, 85, 113, and 141. This study did not establish a maximum tolerated dose for weekly IV treatments with SpA, and with the limited treatment group size, no clear dose-response could be established.

References

1. Langone JJ, Das C, Mainwaring R, Shearer WT. Complexes prepared from protein A and human serum, IgG, or Fc gamma fragments: characterization by immunochemical analysis of ultracentrifugation fractions and studies on their interconversion. 1985 Mol Cell Biochem. Jan;**65**(2):159-70
2. Graille M, Stura E, Corper L, Sutton B, Taussig M, Charbonnier J, et al. Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. 2000 Proc Natl Acad Sci USA;**97**:5399–4
3. MacLellan L, Montgomery J, Sugiyama F, Kistson S, Thummler K, Silverman G, et al. Co-opting endogenous immunoglobulin for the regulation of inflammation and osteoclastogenesis in humans and mice. Arthritis Rheum 2011;**63**:3897-907