Randomised, double-blind, placebo-controlled trial of IL1-trap, rilonacept, in systemic sclerosis. A phase I/II biomarker trial

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ABSTRACT

Objective. *This clinical trial was designed to study the safety and efficacy of blocking IL-1 in skin fibrosis of patients with diffuse cutaneous systemic sclerosis (dcSSc), and to test the hypothesis that inhibition of IL-1 by rilonacept will downregulate expression of the 2G SSc gene biomarker as a surrogate for the modified Rodnan skin score (MRSS).*

Methods. *19 dcSSc patients were randomised 2:1 active treatment:placebo in this double blinded trial. Study patients received weekly treatments with either subcutaneous rilanocept 320 mg loading dose at day 0 and then 160 mg for each of the 5 subsequent weekly doses, or placebo. Skin biopsies were taken to test 2G SSc biomarker gene expression at day 0 before treatment and one week after the final study drug dose, comparing gene expression changes between rilonacept-and placebo-treated patients, as well as the change in gene expression at week 6 compared to baseline in rilonacept-treated patients. Safety assessments extended to 6 weeks after the final dose of study drug or placebo. Other secondary outcome measures included global and IL-1-regulated gene expression, serum biomarkers and the MRSS.*

Results. *Rilonacept compared to placebo-treated patients did not show any treatment-related effect on the 2G SSc biomarker. Rilonacept treatment also failed to alter IL-6 expression in skin, serum IL-6, C-reactive protein, or CCL18, a marker of IL-6 activity in SSc.*

Conclusion. *In this small trial we did not observe any effect of blocking IL-1 on clinical skin disease or biomarkers of IL-1 activity.*

Introduction

Although skin changes in SSc are not a cause of mortality, they cause considerable morbidity, may reflect similar pathological processes to those that occur in the bowel and lungs, and correlate highly with prognosis and disease progression in other organ systems Several observations have implicated the inflammasome and IL-1 in fibrotic diseases, in general, and SSc, specifically. Environmental or occupational exposure to silica dust leads to fibrosis (1) and has been associated with SSc (2). Recently, several groups have shown in murine models that silica dust activates inflammation and fibrosis through the inflammasome (3, 4).

The mechanism linking IL-1 to fibrosis is uncertain, but involves TGF-β/smad3 dependent stimulation (5).

We describe here a short duration (6-week), placebo-controlled clinical trial of the IL-1 inhibitor, rilonacept, utilising skin biomarkers to evaluate drug efficacy. We evaluated the 2G SSc biomarker as the primary outcome measure for this trial, as a surrogate outcome for SSc skin disease.

Materials and methods

Patients and study design

Patients were recruited into the study from the Boston University Medical Center, Hospital of Special Surgery and University of Michigan Medical Center. Informed consent was obtained on all study patients in accordance with the Declaration of Helsinki. All patients met the American College of Rheumatology criteria for SSc with diffuse cutaneous involvement, had a disease duration of <24 months since the onset of the first SSc manifestation other than Raynaud's phenomenon, had a MRSS of ≥15 and were ≥18 years of age. The study was registered with clinicaltrials.gov# NCT01538719 on February 21, 2012.

*Detailed Clinical Trial protocol, and biochemical andstatistical methodology can be found in Supplementary Methods.

Results

Study patients

We enrolled a total of 19 patients with dcSSc. All but two were from Boston University Medical Center and the remaining patients where from the Hospital for Special Surgery and University of Michigan Medical Center. Twelve patients were randomised to receive rilonacept and 7 to receive placebo. The patients in the two groups were of approximately the same age and had about the same MRSS, CRP and SHAQ at study entry (Table I).

Rilonacept-treated patients showed no change in clinical outcomes

The change in the MRSS at each follow-up visit from baseline did not show statistically significant differences between rilonacept- and placebo-treated patients at any time point (Table II). Similar findings were observed when changes in MRSS across follow-up visits from baseline between the two groups were analysed using mixed-effect regression models. The SHAQ was assessed at baseline, week 6 and week 12. At week 12 (6 weeks after stopping the study medication) the placebo-treated group showed a significant decline in SHAQ compared to the rilonacept-treated group. However, no significant difference was noted at the 6 week visit, at the end of treatment.

Biomarkers of skin fibrosis show no change in rilonacept-treated patients

The primary efficacy outcome for this trial was the 2G SSc biomarker. The change in the 2G SSc biomarker at week 6 compared to baseline showed no significant difference between rilonacept and placebo-treated patients.

We hypothesised in a previous trial of anti-TGFβ/fresolimumab that in a short duration, biomarker driven, clinical trial, gene expression is not likely to show a significant spontaneous change after a 3-7 week treatment period, since skin disease in dcSSc is generally seen to change slowly over months to years. We have formally shown this to be the case in a recent trial of a topical C-82 (6). In this study, no change 2G SSc skin biomarker was seen in placebo treated patients after 4 weeks compared to baseline 2G SSc biomarker levels. Therefore, we also compared the 2G SSc biomarker at week 7 compared to baseline in just rilonacept-treated patients (Fig. 1). In contrast to treatment with fresolimumab, but similar to C-82 treated patients, rilonacept-treated patients did not show a change in the 2G SSc biomarker (Supplementary Fig. 1). Thus, this lack of change is similar to that seen after 6-8 weeks in placebo-treated arm of our recently completed C-82 trial (6).

Rilonacept shows no effect on skin gene expression

We carried out a murine experiment in which IL-1 was injected subcutaneously by osmotic pump for one week and altered gene expression examined. Looking at the most highly regulated genes in IL-1 treated murine skin revealed that IL-6 mRNA was highly induced (30-fold) along with a series of other genes (Supplementary Table I).

We examined the skin microarray data, comparing week 6 to baseline gene ex-

Table I. Demographic and baseline characteristics of study participants.

Parameter	Rilonacept (n=12)	Placebo (n=7)	*p*-value
Age			
Mean (SD)	51.33 (7.33)	49.86 (11.10)	0.726
Sex F (%)	7 (58.33)	2 (28.57)	0.349
mRSS			
Mean (SD)	30.41 (8.76)	29.14 (8.93)	0.664
Disease duration (months)			
Mean (SD)	26.06 (11.43)	15.29 (7.89)	0.174
ILD (no. [%])	2 (16.67)	1 (14.29)	>0.999
CRP (mg/l)			
Mean (SD)	13.7 (18.79)	14.8 (18.12)	0.496
Digital ulcers [no. %]	5 (41.67)	1 (14.29)	0.333
SHAQ			
Mean (SD)	1.26 (0.66)	1.45 (0.77)	0.772
Concomitant medications [no. %]			
Corticosteroids	3 (25)	3 (42.86)	0.617
PDE-5 inhibitors	0 (0)	1 (14.29)	>0.999
ACE inhibitors	0 (0)	1 (14.29)	0.368
Calcium Channel Blockers	8 (66.67)	5 (71.43)	>0.999

Means with standard deviations (SD). mRSS: modified Rodnan Skin Score; CRP: C-reactive protein; ILD: interstitial lung disease; SHAQ: Scleroderma-Modified Health Assessment Questionnaire.

Table II. Outcome measures.

	Rilonacept (n=12)	Placebo (n=7)	Difference in means (95% CI)
mRSS			
Visit 2 (week 3)	-1.17	-1.57	0.40 (-2.47, 3.28)
Visit 3 (week 6)	-0.67	-0.40 (5)	-0.27 (-6.18, 5.65)
Visit 4 (week 12)	1.33	0.60 (5)	0.73 (-4.17, 5.63)
Difference in mRSS over time (95% CI)*	-0.17	-0.59	0.37 (-2.52, 3.27)
SHAQ			
Visit 3 (week 6)	0.198	-0.075 (4)	0.273 (-0.199, 0.745)
Visit 4 (week 12)	0.219	-0.10 (5)	0.319 (0.045, 0.593)**
Difference in SHAQ over time (95% CI)*	0.208	-0.088	0.296 (-0.069, 0.661)
CRP (mg/L)***			
Visit 2 (week 3)	-7.34 (11)	-9.88 (5)	2.54 (-14.28, 19.36)
Visit 3 (week 6)	-1.67 (11)	-5.55 (4)	3.88 (-21.64, 29.39)
Difference in CRP over time (95% CI)*	-4.63	-7.96	2.96 (-12.49, 18.40)

Changes in MRSS, SHAQ and CRP between rilonacept and placebo groups examined by mix-effects regression modelling and adjusting for time.
*adjusted for time; **$p<0.05$; ***difference from screening visit.

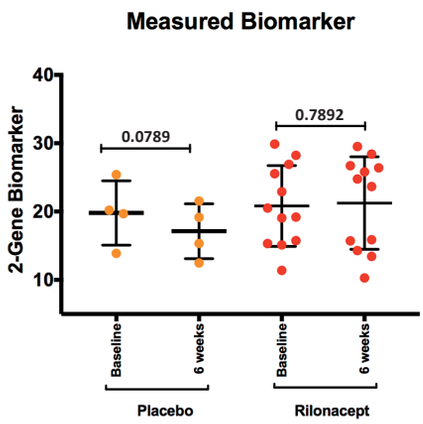

Fig. 1. Rilonacept treatment does not show improvements in the 2-gene biomarker.
PBO: placebo; RT: Rilonacept; w: week.

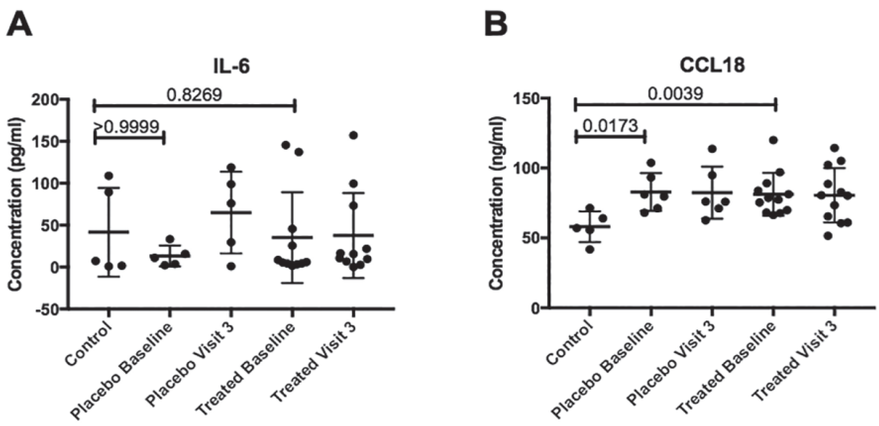

Fig. 2. IL-6 and CCL18 serum concentrations.
A: IL-6 serum levels comparison between control, placebo and treatement groups.
B: CCL18 serum levels in control, placebo and rilonacept treated subjects.

pression in rilonacept-treated patient of the 25 genes most highly induced by IL-1 in murine skin (Supplementary Table I). Although several of these genes showed a trend in uncorrected *p*-values, no consistent effect of blocking IL-1 on skin gene expression was apparent. We also examined Reactome and BioCarta gene sets associated with IL-1 signalling and observed no significant differences in gene expression (Supplementary Tables II and III).

Serum biomarkers are not altered by rilonacept

CRP is upregulated by IL-6, but is also synergistically induced by IL-1 (7). The change in CRP from baseline, at weeks 2 and 6, showed no difference between rilonacept and placebo-treated patients (Table II).

Several, though not all, studies have shown that interleukin-6 (IL-6) is elevated in the serum of some SSc patients (8-11). Interleukin-1 strongly induces IL-6 in SSc fibroblasts (12), and a recent study has suggested that inhibiting IL-6 provides some benefit for SSc skin disease (13). We therefore measured IL-6 levels in the sera of study patients as a potential marker of IL-1 bioactivity. IL-6 levels were highly variable, consistent with other reports and showed no consistent increase in patients compared to several control sera tested (Fig. 2A).

The anti-IL6/tocilizumab study demonstrated that CCL18/PARC, which is elevated in SSc patients, decreased rapidly and to nearly normal levels in tocilizumab, but not placebo-treated patients (13). Thus, we anticipated that if IL-1 was responsible for increased IL-6 in SSc patients, then we could better assess this be measuring CCL18. We found that CCL18 was significantly increased in both our rilonacept and placebo-treated patients (Fig. 2B). CCL18 levels showed no change from baseline in rilonacept- or placebo-treated patients.

Adverse events

We observed few adverse events. Generally, these were expected such as injection site reactions, allergic reaction (Supplementary Table IV). Two patients developed self-limited infections, one a subcutaneous abscess and one a biopsy site infection. One patient each developed transient left breast swelling, diarrhoea, worsening digital ulcer and allergic skin eruption.

Discussion

In this small controlled study we were not able to detect a significant effect of rilonacept on several measures. We did not detect an effect on the 2G SSc biomarker, in the MRSS, or in global skin gene expression. Also, notably, we did not detect any change in skin IL6 mRNA expression, serum IL-6 levels or in CCL18 an apparent surrogate for IL-6 in the tolcilizumab study. Together these data strongly argue against an important effect for IL-1 in SSc.

A limitation of this study is the relatively short duration of treatment. The 6-week treatment period was chosen to permit efficient opportunity to assess the effect of blocking IL-1 without requiring patients to potentially be on placebo for an extended period of time. We anticipated that we might detect changes in IL-1 target genes relatively soon after treatment. Indeed, in the study of tocilizumab in SSc, CCL18 fell dramatically at the first follow-up visit after only 3 weeks of treatment (13). The lack of effect on this biomarker appears to be our strongest evidence that IL-1 is not playing a key role in pathogenesis of most patients with dcSSc. Another limitation of this study is that it is underpowered. As a phase I/II study, it was powered to require a large effect size (~1.3) in order to see a significant difference between groups. Incomplete recruitment and dropouts in the placebo arm further weakened the power to see differences.

In contrast to the lack of effect on biomarkers, the lack of clinical effect of skin disease as assessed by the MRSS over the same period of time is less meaningful, since manifesting changes in fibrotic skin might take longer. Since the effect of tocilizumab on skin disease was seen after longer periods of treatment, 6 and 12 months (13), we cannot exclude the possibility that blocking IL-1 over a longer period of time might lead to an effect on skin.

IL-1 and IL-6 can each be upregulated by a variety of stimuli. We have previously shown that mice treated subcutaneously with the TLR4 agonist, lipopolysaccaride (LPS), show highly upregulated expression of both IL-1

and IL-6, and skin fibrosis (14). However, it seems that in dcSSc skin IL-6, but not IL-1 is likely playing a role. To the extent that murine skin reflects processes occurring in human skin these results suggest that neither TLR4 nor IL-1 are mediating IL-6 upregulation in SSc skin.

In summary, we have tested the effect of blocking IL-1 in patients with dcSSc. After careful evaluation of biomarkers we find no evidence for expected changes in biomarker gene expression or protein levels.

Key messages

- Blocking interleukin-1 did not affect clinical skin disease in patients with systemic sclerosis.
- Rilonacept did not alter 2G SSc biomarker, or biomarkers of interleukin-1 or interleukin-6.

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