ACR Open Rheumatology
Vol. 1, No. 7, September 2019, pp 403–411
DOI 10.1002/acr2.11049

American College of Rheumatology
Empowering Rheumatology Professionals

Safety and Tolerability of Thrombin Inhibition in Scleroderma-Associated Interstitial Lung Disease

R. M. Silver,[1] D. A. Wilson,[1] T. Akter,[1] I. Atanelishvili,[1] J. T. Huggins,[1] K. Kajdasz,[1] K. B. Highland,[2] P. J. Nietert,[1] and G. S. Bogatkevich[1]

Objective. Interstitial lung disease (ILD) is a frequent complication of systemic sclerosis (SSc) (scleroderma) and the leading cause of scleroderma-related deaths. There exists an unmet need for a new drug therapy for ILD-complicated SSc. Substantial evidence supports an important role for thrombin in the pathogenesis of SSc-associated ILD (hereafter SSc-ILD), and targeting thrombin with a direct thrombin inhibitor could prove to be a novel and effective treatment strategy. As a first step toward designing a clinical trial to test the efficacy of thrombin inhibition in SSc-ILD, we conducted this study to test the safety and tolerability of dabigatran in patients with SSc-ILD.

Methods. We performed a prospective, single-center, open-label treatment trial with the direct thrombin inhibitor, dabigatran, in patients with SSc-ILD. Any patient with a history of gastrointestinal hemorrhage or gastric antral vascular ectasia was excluded. Blood monitoring was performed monthly, and patient-reported outcomes, pulmonary function tests, and skin scores were obtained at baseline and at 3- and 6-month visits. Bronchoscopy with bronchoalveolar lavage (BAL) was performed at baseline and at 6 months for measurement of lung thrombin activity.

Results. Of 15 patients with SSc-ILD, 14 completed 6 months of treatment with dabigatran at 75 mg taken orally twice daily. Adverse events were uncommon and usually mild or unrelated to the study medication. No serious adverse event was observed. Dabigatran was well tolerated, and we observed no significant gastrointestinal, pulmonary, or other safety issues or intolerability. BAL fluid thrombin activity decreased or remained stable in 13 of 14 (92.8%) subjects.

Conclusion. Dabigatran appears to be safe and well tolerated in patients with SSc-ILD. A larger randomized controlled trial to test the efficacy of direct thrombin inhibition with dabigatran can be considered.

INTRODUCTION

Systemic sclerosis (SSc) (scleroderma) is a multisystem connective-tissue disease characterized by microvascular injury, autoimmunity, and fibrosis (1). Pulmonary involvement occurs frequently in patients with SSc, and interstitial lung disease (ILD) is the leading cause of SSc-related deaths (2). Despite the substantial disease burden associated with SSc-associated ILD (hereafter SSc-ILD), there is currently no US Food and Drug Administration (FDA)–approved medication for treating this condition (3).

Substantial data implicate the serine protease thrombin in the pathogenesis of SSc-ILD as well as in other ILDs, for example, idiopathic pulmonary fibrosis (IPF) (4–6). Lung injury, when coupled with increased vascular permeability, leads to extravasation of plasma-clotting factors into injured airspaces, where exposure to tissue factor initiates the coagulation cascade and the generation of thrombin and fibrin (4,5). Thrombin, in turn, promotes lung fibrosis through interaction with specific cell-surface receptors (4,5,7).

Thrombin inhibition results in attenuation of lung fibrosis in vitro and in in vivo animal experiments, leading others and us to propose thrombin inhibition as an attractive strategy for the treatment of SSc-ILD and other fibrosing lung diseases (5,8,9). Whereas general inhibition of the coagulation cascade in patients with lung fibrosis may be detrimental (10), inhibiting thrombin's fibrotic properties while avoiding a significant effect on hemostasis could have important clinical implications (5,11,12). As a first step toward testing a strategy of thrombin inhibition in a patient population inherently at increased risk of gastrointestinal bleeding, we sought to determine whether treatment of patients with SSc-ILD

ClinicalTrials.gov identifier: NCT02426229.

Dr. Silver's work was supported by an award from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH (1-R21-AR-065089-01) and by an award from the Scleroderma Foundation.

[1]R. M. Silver, MD, D. A. Wilson, PhD, T. Akter, PhD, I. Atanelishvili, MS, J. T. Huggins, MD, K. Kajdasz, P. J. Nietert, PhD, G. S, Bogatkevich, MD, PhD: Medical University of South Carolina, Charleston; [2]K. B. Highland, MD: Cleveland Clinic, Cleveland, Ohio.

No potential conflicts of interest relevant to this article were reported.

Address correspondence to R. M. Silver, MD, Division of Rheumatology and Immunology, Medical University of South Carolina, 96 Jonathan Lucas Street, Suite 822, Charleston, SC 29425. E-mail: silverr@musc.edu.

Submitted for publication March 25, 2019; accepted in revised form June 12, 2019.

Table 1. Baseline characteristics of the study cohort

Patient	Sex	Race	Age	SSc Classification	ANA[a]	Disease Duration	FVC (l), %Ref	FEV_1 (l), %Ref	DLCO, (ml/min/mm Hg), %Ref	Mahler Dyspnea Index	HRCT Chest Scan	Rx	Duration (mo)	Prior Therapy
1	Female	White	41.3	lcSSc	PMScl	7.75	58	60	45	7	NSIP	MMF	53	…
2	Female	Black	50.6	dcSSc	Scl70[b]	6.90	41	47	37	14	NSIP	MMF	54	…
3	Male	White	49.1	dcSSc	Speck	1.58	51	60	39	6	UIP	MMF	1	…
4	Female	White	45	lcSSc	Scl70[b]	4.00	75	76	57	4	NSIP	…	…	…
5	Female	Black	45.2	dcSSc	Scl70[b]	9.08	65	61	42	5	NSIP	…		…
6	Female	Black	44.2	lcSSc	Scl70[b]	10.00	55	62	52	8	UIP	MMF	28	…
7	Female	White	66.1	lcSSc	Scl70[b]	2.33	82	77	60	8	NSIP	…	…	…
8	Male	White	56.7	dcSSc	Scl70[b]	4.58	75	83	55	7	NSIP	MMF	35	Cyclophosphamide
9	Female	White	57.6	dcSSc	Scl70[b]	0.92	82	76	55	4	UIP	Ritux	…	…
10	Female	Black	35.7	lcSSc	Nucleo	3.00	84	88	86	10	NSIP	MMF	29	…
11	Female	White	42	lcSSc	PMScl	5.25	99	94	64	10	NSIP	MMF	50	Cyclophosphamide
12	Female	Black	30.7	dcSSc	Speck	1.58	70	66	63	14	UIP	MMF	9	…
13	Female	Black	40	dcSSc	Nucleo	1.33	83	80	69	5	…	MMF	12	Tocilizumab
14	Female	White	60.6	dcSSc	Scl70[b]	4.17	60	67	31	8	NSIP	MMF	7	…
15	Female	White	48.2	lcSSc	Scl70[b]	2.25	79	78	59	8	NSIP	MMF	16	…
Total	13 female and 2 male	9 white and 6 black	47.5 ± 9.6	7 lcSSc and 8 dcSSc	…	4.3 ± 2.9	70.6 ± 15.4	71.7 ± 12.6	54.3 ± 14.1	7.86 ± 3.11	…	…	…	…

Abbreviation: ANA, antinuclear antibody; dcSSc, diffuse cutaneous systemic sclerosis; DLCO, diffusion capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HRCT, high-resolution computed tomography; lcSSc, limited cutaneous systemic sclerosis; MMF, mycophenolate mofetil; nucleo, nucleolar antinuclear antibody pattern; NSIP, nonspecific interstitial pneumonia; Ritux, rituximab; Rx, Treatment; speck, speckled antinuclear antibody pattern; UIP, usual interstitial pneumonia; %Ref, predicted.
[a]ANAs include PMScl and anti-PM/Scl antibodies.
[b]Anti-topoisomerase antibodies.

with a direct thrombin inhibitor, dabigatran, would be safe and well tolerated.

PATIENTS AND METHODS

To test the prediction that dabigatran (dabigatran etexilate) is safe, we conducted a prospective, single-center, open-label treatment trial with a specific focus on the incidence of bleeding and gastrointestinal side effects in 15 patients with SSc-ILD treated for 6 months at a dose of 75 mg taken orally twice daily. Because patients with SSc-ILD may potentially have a greater risk of bleeding and dyspepsia than the population with atrial fibrillation studied in the pivotal trials for dabigatran etexilate, we chose the lowest available dose for this safety study (75 mg twice daily). The study was approved by the Institutional Review Board of the Medical University of South Carolina and by a data and safety monitoring board (DSMB) established on behalf of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH).

Recruitment strategy, eligibility, schedule of study visits, assessments, study-related procedures, data collection, and study forms are detailed in a manual of procedures developed by the investigators and approved by the DSMB (see Supplemental Material). Potential study subjects were recruited from the Scleroderma Clinic at the Medical University of South Carolina. Entry criteria included the following: 1) age between 18 and 70 years, 2) fulfillment of the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for SSc, 3) SSc of less than 10 years in duration (with the onset of SSc defined as the date of the first non-Raynaud phenomenon manifestation), 4) presence of ILD (defined as any ground glass on the high-resolution computed tomography [HRCT] chest scan and more than 20% involvement by pulmonary fibrosis and/or a predicted forced vital capacity [FVC] of less than 70%), and 5) stable dosage of background immunosuppressive medication for at least 6 weeks prior to enrollment.

Patients were excluded from enrolling if any of the following conditions was present: 1) predicted FVC of less than 40% and/or predicted diffusion capacity for carbon monoxide (DLCO) of less than 30%, 2) clinically significant pulmonary hypertension requiring treatment based on the clinician's judgement, 3) smoking within 3 months prior to enrollment, and 4) risk of bleeding (eg, current anticoagulation, aspirin therapy, platelet count of less than 100,000 per mm^3, and history of gastrointestinal hemorrhage or gastric antral vascular ectasia). Out of an abundance of caution, our study was designed to enroll no more than 3 patients at a time, with at least 1 month between the last of each group of 3 and the first of the next group of 3. The purpose of the waiting time, along with a set of stopping rules approved by the DSMB, was to minimize the chance of multiple subjects being exposed to the study drug should it be proven not to be safe.

Laboratory studies that included complete blood cell count (CBC), a comprehensive metabolic profile (CMP), and coagulation tests (prothrombin time [PT], partial thromboplastin time [PTT], and thrombin time [TT]) were performed at the baseline study visit and then monthly for the duration of the study. Bronchoscopy with bronchoalveolar lavage (BAL) was performed at baseline and at the end of the study (6 months) by a trained pulmonologist (JTH), as previously described (13). Thrombin activity was measured in BAL fluid by using a spectrophotometric method (14). Patient-reported outcomes were assessed by using the Mahler Dyspnea Baseline and Transitional Index (15,16), the Scleroderma Health Assessment Questionnaire (SHAQ) (17), and the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract 2.0 Instrument (GIT) (18) at the baseline, 3-month, and 6-month visits.

Because the focus of this trial was safety, the primary analysis was a sequential probability ratio test (19) that would have resulted in early stopping of the study had the major complication rate exceeded 5%. Exploratory analyses included calculating descriptive statistics for clinical measures at baseline and over time. Linear mixed models with random-subject effects were used to determine whether the changes in clinical measures over the 6-month study period were statistically significant. At each follow-up time point, P values for the change in mean from baseline were also generated by using paired t tests or Wilcoxon signed-rank tests as appropriate. For the exploratory analyses, the α level was set to 0.10, meaning that $P < 0.10$ was considered statistically significant. All analyses were performed with SAS version 9.4. (SAS Institute, Inc).

RESULTS

Patient cohort. Between March 2016 and December 2017, 15 subjects with SSc-ILD were enrolled in a single-center open-label treatment trial to test the safety and tolerability of dabigatran at a dose of 75 mg taken orally twice daily. The baseline demographic and pulmonary features of the patients are shown in Table 1. A majority of subjects were white women (87%) with a mean age of 47.5 ± 9.6 years and a mean disease duration of 4.3 ± 2.9 years. Most study subjects had an autoantibody known to be associated with an increased risk for SSc-ILD: Scl-70 (9 of 15; 60%) or PM/Scl (2 of 15; 13%). All had dyspnea (Mahler Dyspnea Index of 4-14; mean: 7.86 ± 3.11), and most had a restrictive pattern on the pulmonary function test (10 of 15; 67%), with a nonspecific interstitial pneumonia pattern on the HRCT chest scan (10 of 15; 67%). Most were receiving mycophenolate mofetil (MMF) as the treatment of SSc-ILD (11 of 15; 73%) (20). Of the 15 subjects, 14 (93.3%) completed the 6-month treatment protocol. One subject (patient 10; Table 1) was excluded for inability to comply with study protocol visits.

Table 2. Listing of adverse events

Patient	Event	Relationship	Intervention Discontinued	Severity	Outcome	Serious
3	Fatigue	Not related	No	Mild	Unresolved	No
5	Menorrhagia	Possibly	No	Mild	Resolved	No
5	Headache	Not related	No	Mild	Unresolved	No
6	Chiari malformation	Not related	No	Moderate	Resolved	No
7	Epistaxis	Possibly	No	Mild	Resolved	No
7	Cystitis	Not related	No	Moderate	Resolved	No
7	Epistaxis	Possibly	No	Mild	Resolved	No
9	Epistaxis	Possibly	No	Mild	Resolved	No
14	Epistaxis	Possibly	No	Mild	Resolved	No
15	Menorrhagia	Possibly	No	Mild	Resolved	No
15	Contact dermatitis	Not related	No	Moderate	Unresolved	No

Safety of dabigatran. Of the 14 subjects who completed the 6-month treatment protocol, 7 patients experienced a total of 11 adverse events (AEs) (Table 2). AEs included fatigue, headache, menorrhagia, and epistaxis. All AEs were graded as mild, except for one moderate-grade AE (headache), which the study team and DSMB members felt was not related to the study drug (headache attributed to a preexisting Chiari malformation that resolved after a neurosurgical procedure that required a brief interruption of the study drug). No serious adverse event (SAE) was recorded for any of the study subjects; thus, the sequential probability ratio test never yielded an indication that the SAE event rate was greater than 5%. Monthly laboratory monitoring of CBC and CMP showed no signs of drug toxicity. No significant change in hemoglobin or hematocrit levels was observed. Coagulation studies (Table 3) demonstrated no significant change in the PT. The mean PTT was increased (5.1 ± 4.9 seconds; $P < 0.004$), which was not felt to be clinically significant. Prolongation of the plasma TT was observed in 6 of 9 (67%) of subjects, for whom baseline and 6-month data were available. All data were periodically reviewed by the DSMB, which found no significant safety issues and no reason to invoke the stopping rules set forth in the Manual of Procedures (MOP).

The mean pulmonary function measures for the group did not change significantly over time (Table 4, Figure 1); however, some individuals did experience declines greater than 10% in FVC, forced expiratory volume (FEV), or DLCO (n = 4) or improvements greater than 10% (n = 4) from baseline to the end of the study. Results from the linear mixed models confirmed that there was no significant change in pulmonary function over time ($P = 0.24$, $P = 0.24$, and $P = 0.79$ for predicted FVC percentage, predicted forced expiratory volume in 1 second (FEV_1) percentage, and predicted DLCO percentage, respectively). Consistent with the findings on the pulmonary function test, we found no significant change in self-reported dyspnea during the 6-month treatment period ($P = 0.41$). Of 14 subjects, 4 reported worsening dyspnea, 3 reported no change, and 6 reported improvement.

Tolerability of dabigatran. Dabigatran in a dose of 75 mg taken orally twice daily was well tolerated. We observed no patient dropout due to intolerance or AE. Table 5 lists GIT and SHAQ scores and the modified Rodnan Skin Score (mRSS) at the various study time points, and they are displayed in Figure 2. The 3-month and 6-month GIT assessments showed no significant change from baseline GIT scores, and a linear mixed model confirmed that there was no significant ($P = 0.45$) change over time in GIT scores. No significant change was noted in the SHAQ score at individual time points or overall during treatment with dabigatran ($P = 0.89$ from the linear mixed model). The mRSS declined (improved) by the 6-month visit compared with

Table 3. Coagulation studies at baseline and at the 6-mo visit

	Baseline	6-mo Visit	Change	P[a]
Prothrombin time (mean ± SD), s (normal range: 9.1-12.0)	13.8 ± 1.2	14.4 ± 1.3	0.7 ± 1.4	0.10
Partial thromboplastin time (mean ± SD), s (normal range: 24-33)	30.6 ± 2.8	35.8 ± 4.9	5.1 ± 4.9	0.004
Thrombin time, No. elevated (% elevated)[b]	1 (7.7)	8 (50.0)	6 (66.7)	0.03

[a] P for change from baseline.
[b] Elevated is defined as > 20 s.

Table 4. Pulmonary function tests

				Change From Baseline	
	Study Visits	N	Mean ± SD	Mean± SD	*P*
FVC, % predicted	Baseline	15	70.6 ± 15.4	...	...
	3-mo visit	14	70.0 ± 15.4	0.4 ± 9.9	0.89
	6-mo visit	14	67.3 ± 13.6	-2.4 ± 5.9	0.16
FEV_1, % predicted	Baseline	15	71.7 ± 12.6	...	...
	3-mo visit	14	70.3 ± 11.6	−0.2 ± 9.5	0.93
	6-mo visit	14	68.4 ± 9.3	−2.1 ± 6.0	0.22
DLCO, % predicted	Baseline	15	54.3 ± 14.1	...	...
	3-mo visit	14	52.3 ± 14.2	0.3 ± 6.4	0.87
	6-mo visit	14	52.6 ± 11.5	0.6 ± 5.0	0.68

Abbreviation: DLCO, diffusion capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

the mRSS at baseline. Results from a linear mixed model indicated that the average monthly decline in mRSS was 1.1 units (95% confidence interval [CI]: 0.4-1.7 units; $P = 0.004$).

BAL fluid thrombin activity. BAL fluid thrombin activity was elevated (>6.4 ng/ml) at baseline in only 6 of 14 of our study subjects (43%) (Table 6). After treatment with dabigatran, mean thrombin activity was not significantly different from that at baseline, with reduced thrombin activity in 5 of 6 of those with elevated thrombin activity at baseline (83.3%) and a return to the normal range in 4 of 6 (66.7%). One subject (patient 12; Table 1) had a substantial increase in BAL fluid thrombin activity after 6 months of therapy with dabigatran. Excluding this single case, the BAL fluid thrombin activity decreased or remained stable in 13 of 14 (92.8%) subjects.

DISCUSSION

SSc-ILD is a major cause of morbidity and mortality for which no FDA-approved treatment exists (2,3). Cyclophosphamide (2), MMF(20), and myeloablative autologous stem-cell transplantation (21) have been shown to improve lung function in a variable percentage of patients, but such therapies are often toxic or ineffective. Two FDA-approved drugs for IPF, nintedanib and pirfenidone, and a number of other drugs are currently under investigation (3). Given the substantial disease burden and unmet need, there is an urgency for novel treatment strategies for SSc-ILDs.

One such strategy involves targeting the serine protease thrombin. The rationale for this strategy is based on extensive evidence showing that thrombin plays an important role in the

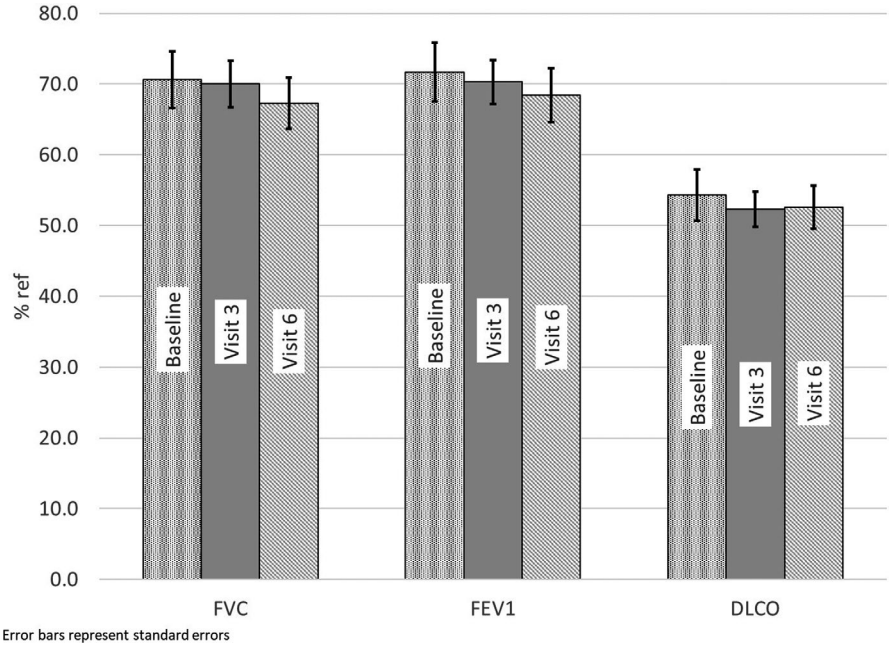

Figure 1. Change from baseline pulmonary function tests at 3 and 6 months (all expressed as percentage predicted). Error bars represent SEM. DLCO, diffusing capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; %ref, predicted.

Table 5. mRSS, SHAQ score, and GIT score

				Change From Baseline		
Questionnaire	Study Visits	N	Mean± SD	N	Mean± SD	*P*
mRSS (range: 0-51)	Baseline	15	16 ± 10.3		...	...
	3-mo visit	14	12 ± 8.8	14	−5.1 ± 6.2	0.009
	6-mo visit	14	10.5 ± 8.5	14	−6.6 ± 6.4	0.002
SHAQ (range: 0-3)	Baseline	15	1.4 ± 0.6		...	...
	3-mo visit	14	1.5 ± 0.7	14	0.2 ± 0.3	0.07
	6-mo visit	14	1.4 ± 0.7	14	0.0 ± 0.3	0.80
Gastrointestinal symptoms (GIT) (range: 0-3)	Baseline	15	0.8 ± 0.6		...	...
	3-mo visit	14	0.7 ± 0.6	13	−0.0 ± 0.2	0.76
	6-mo visit	14	0.7 ± 0.6	14	−0.0 ± 0.2	0.70

Abbreviation: GIT, University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract2.0 Instrument; mRSS, Modified Rodnan Skin Score, SHAQ, Scleroderma Health Assessment Questionnaire.

pathogenesis of SSc-ILD as well as IPF (see below). To assess potential risks of thrombin inhibition in patients with SSc-ILD, we conducted a pilot study in which 14 of 15 patients with SSc-ILD completed 6 months of treatment with dabigatran (75 mg taken orally twice daily). The demographics of our study population are similar to those of Scleroderma Lung Study I (SLS I) and Scleroderma Lung Study II (SLS II) (20,22), although the disease duration of our study population was somewhat longer. AEs were uncommon and were usually mild or unrelated to the study medication. There were no SAEs. Dabigatran was well tolerated, and we observed no significant gastrointestinal, pulmonary, or other safety issues or intolerability.

The primary aim of our study was to assess safety. Given the small cohort size, this study was not powered to assess efficacy. We did, however, obtain pulmonary function testing at the baseline, 3-month, and 6-month visits out of an abundance of caution because systemic anticoagulation with warfarin in a prior IPF clinical trial was found to be associated with an increase in risk of FVC decline and patient death (10). With dabigatran treatment, we observed no significant deterioration of pulmonary function or dyspnea at the 3-month or 6-month assessments, suggesting that, unlike warfarin, dabigatran is not associated with an increased risk of respiratory decline. The mean pulmonary function measures for the group did not change significantly over time (Table 4, Figure 1); however, some individuals did experience declines greater than 10% in FVC, FEV, or DLCO (n = 4) or improvements greater than 10% (n = 4) from baseline to the end of the study.

To assess gastrointestinal tolerability, patients completed the GIT, a validated self-administered 34-item tool that captures the gastrointestinal burden associated with SSc as well as the majority of the commonly reported gastrointestinal symptoms

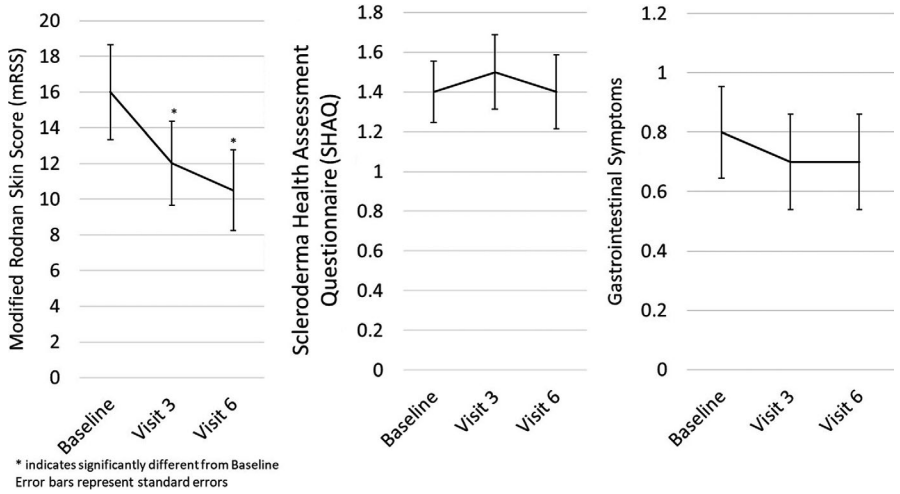

Figure 2. Clinical outcomes at baseline, 3 months, and 6 months for the modified Rodnan Skin Score (mRSS), the Scleroderma Health Assessment Questionnaire (SHAQ), and the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract 2.0 Instrument. Error bars represent SEM. [a]Significantly different from baseline.

Table 6. BAL thrombin activity

Patient	Baseline (ng/ml)	6 mo (ng/ml)	Absolute Difference	% Difference
1	6.29	7.24	0.94	0.15
2	4.06	4.30	0.24	0.06
3	31.95	5.06	−26.89	−0.84
4	5.59	4.56	−1.03	−0.18
5	9.76	4.63	−5.13	−0.53
6	4.01	3.46	−0.55	−0.14
7	9.80	10.33	0.54	0.05
8	11.33	7.59	−3.75	−0.33
9	15.33	4.75	−10.58	−0.69
11	5.29	6.21	0.92	0.17
12	6.10	100.18	94.08	15.43
13	4.18	5.89	1.71	0.41
14	6.99	4.69	−2.30	−0.33
15	4.15	4.95	0.81	0.19
Median (IQR)	6.2 (4.2 to 9.8)	5.0 (4.6 to 7.2)	−0.2 (−3.8 to 0.9) (NS)	−0.05 (−0.3 to 0.2) (NS)
Median (IQR) (excluding patient No. 12)	6.3 (4.2 to 9.8)	5.0 (4.6 to 6.2)	0.6 (−3.8 to 0.8) (NS)	−0.1 (−0.3 to 0.2) (NS)

Abbreviation: IQR, interquartile range; NS, not statistically significant.

associated with dabigatran (18). GIT assessments indicated no significant change in gastrointestinal symptoms. Patients also completed the SHAQ, a widely used and well-characterized outcome measure for SSc (17). No significant change was noted in the SHAQ score at individual time points or overall during treatment with dabigatran, indicating an absence of any negative effect of treatment on overall SSc disease status. The mRSS declined at the 6-month visit compared with the baseline visit, with an average monthly decline in mRSS of 1.1 units (95% CI: 0.4-1.7 units; $P = 0.004$). Given the small sample size and the lack of a control group, no conclusions can be drawn regarding an effect of dabigatran on the mRSS.

There is substantial evidence supporting vascular injury and overexpression of thrombin in the pathogenesis of both SSc-ILD and IPF (8,14,23–30). We previously reported that thrombin activity is highly expressed in the BAL fluid of patients with SSc-ILD (14). Additionally, we observed that after a brief exposure to thrombin in vitro, normal lung fibroblasts undergo differentiation to a myofibroblast phenotype, with such activation occurring via a protease activated receptor-1 (PAR-1)/protein kinase C-epsilon (PKCε)-dependent pathway (28). Dabigatran inhibits thrombin by preventing cleavage of the extracellular domain of the PAR-1 receptor, thereby inhibiting thrombin's cellular effects (28,29). We have shown that dabigatran inhibits thrombin-induced differentiation of normal lung fibroblasts to the SSc-like myofibroblast phenotype, and with thrombin inhibition, SSc lung fibroblasts exhibit decreased expression of connective tissue growth factor (CTGF) (*CCN2*), α-smooth muscle actin, and collagen type I (28,29). We also demonstrated that dabigatran has anti-inflammatory and antifibrotic effects in a bleomycin model of pulmonary fibrosis, and no hemorrhagic complication was observed with such treatment (8). Thus, we and others have concluded that the antifibrotic effects of dabigatran are specific to inhibition of thrombin per se, rather than to its less-specific effects on coagulation and hemostasis, and thus the dissociation of the antifibrotic effects from hemostasis may have important clinical implications (5,11).

BAL fluid thrombin activity was elevated at the baseline assessment in only 6 of 14 of our study subjects (43%), perhaps owing to the frequent use of immunosuppressive therapy in these patients. After treatment with dabigatran, the mean thrombin activity was not significantly different from that at baseline. A single subject was found to have a substantial increase in BAL fluid thrombin activity at the 6-month visit. We do not have a definitive explanation for this increase; however, we can exclude noncompliance because the plasma TT measured at the time of the BAL procedure was more than 50 seconds. This patient's overall disease may have been more active, because the mRSS had increased, dyspnea had worsened, and the BAL cell count differential contained 10% neutrophils. Excluding this single case, the BAL fluid thrombin activity decreased or remained stable in 13 of 14 (92.8%) subjects.

For those patients whose BAL fluid thrombin expression was elevated at baseline, dabigatran administration lowered thrombin activity. In some of our study subjects, the BAL fluid thrombin activity was normal at baseline. Future studies might employ a thrombin activity measurement for cohort enrichment, electing to treat only those patients with increased lung thrombin activity. A low dose of 75 mg twice daily was chosen for

this exploratory study because no previous safety studies had been reported in this patient group; however, some patients may require a higher dosage (eg, 150 mg twice daily) to maintain low expression of lung thrombin activity. Future studies should be designed to explore this issue as well as to further explore the safety and the efficacy of dabigatran in patients with SSc-ILD and other fibrosing lung diseases.

ACKNOWLEDGMENTS

The authors thank Carol A. Lambourne, MSc, PhD, for assistance in developing the MOP. The authors also wish to thank the members of the staff at KAI, Inc; James Witter, MD, PhD and the staff at the NIAMS; and the DSMB (Eliza Chakravarty, MD, Chairperson; Thomas S. Kickler, MD, DSMB Safety Officer; and Weng Kee Wong, PhD, MS, Statistician) for their time and efforts to ensure the successful and safe conduct of this study.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Silver, Highland, Nietert, Bogatkevich.
Acquisition of data. Silver, Wilson, Akter, Atanelishvili, Huggins, Kajdasz, Highland, Nietert, Bogatkevich.
Analysis and interpretation of data. Silver, Wilson, Akter, Atanelishvili, Huggins, Kajdasz, Highland, Nietert, Bogatkevich.

REFERENCES

1. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities. J Scleroderma Relat Disord 2017;2:137–52.
2. Tyndall AJ, Bannert B, Vonk M, Airò P, Cozzi F, Carreira PE, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. Ann Rheum Dis 2010;69:1809–15.
3. Volkmann ER, Tashkin DP. Treatment of systemic sclerosis-related interstitial lung disease: a review of existing and emerging therapies. Ann Amer Thor Soc 2016;13:2045–56.
4. Ludwicka-Bradley A, Silver RM, Bogatkevich GS. Coagulation and autoimmunity in scleroderma interstitial lung disease. Semin Arthritis Rheum 2011;41:212–22.
5. Shea BS, Probst CK, Brazee PL, Rotile NJ, Blasi F, Weinreb PH, et al. Uncoupling of the profibrotic and hemostatic effects of thrombin in lung fibrosis. JCI Insight 2017;2:86608.
6. Lumpkin TB, Southern BD, Scheraga RG, Crish JF, Grove L, Abraham S, et al. Direct thrombin inhibition with dabigatran alters the matrix-induced, pro-fibrotic phenotype of fibroblasts. Am J Respir Crit Care Med 2018;197:A5794.
7. Bogatkevich GS, Gustilo E, Oates JC, Feghali-Bostwick C, Harley RA, Silver RM, et al. Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. Am J Physiol Lung Cell Mol Physiol 2005;288:L190–201.
8. Bogatkevich GS, Ludwicka-Bradley A, Nietert PJ, Akter T, van Ryn J, Silver RM. Antiinflammatory and antifibrotic effects of the oral direct thrombin inhibitor dabigatran etexilate in a murine model of interstitial lung disease. Arthritis Rheum 2011;63:1416–25.
9. Leask A. Thrombin-induced CCN2 expression as a target for anti-fibrotic therapy in scleroderma. J Cell Commun Signal 2010;4:111–2.
10. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2012;186:88–95.
11. Bogatkevich GS, Nietert PJ, Silver RM, Highland KB. Rationale for anticoagulant therapy of pulmonary fibrosis. Am J Respir Crit Care Med 2014;189:362–3.
12. Noth I, Olman MA. Reply: rationale for anticoagulant therapy of pulmonary fibrosis [letter]. Am J Respir Crit Care Med 2014;189:363.
13. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. Am Rev Respir Dis 1990;141(Pt 2):S169–202.
14. Ohba T, McDonald JK, Silver RM, Strange C, LeRoy EC, Ludwicka A. Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation via induction of the platelet-derived growth factor α-receptor. Am J Respir Cell Mol Biol 1994;10:405–12.
15. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest 1984;85:751–8.
16. Mahler DA, Waterman LA, Ward J, McCusker C, ZuWallack R, Baird JC. Validity and responsiveness of the self-administered computerized versions of the baseline and transition dyspnea indexes. Chest 2007;132:1283–90.
17. Pope J. Measures of systemic sclerosis (scleroderma). Arthritis Care Res (Hoboken) 2011;63 Suppl:S98–111.
18. Khanna D, Hays RD, Maranian P, Seibold JR, Impens A, Mayes MD, et al. Reliability and validity of the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument. Arthritis Rheum 2009;61:1257–63.
19. Wald A. Sequential tests of statistical hypothesis. The Annals Mathematical Statistics 1945;16:117–86.
20. Tashkin DP, Roth MD, Clements PJ, Furst DE, Khanna D, Kleerup EC, et al. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomized controlled, double-blind, parallel group trial. Lancet Respir Med 2016;4:708–19.
21. Sullivan KM, Goldmuntz EA, Keyes-Elstein L, McSweeney PA, Pinckney A, Welch B, et al. Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med 2018;378:35–47.
22. Tashkin DP, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, et al. Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med 2006;354:2655–66.
23. Harrison NK, Glanville AR, Strickland B, Haslam PL, Corrin B, Addis BJ, et al. Pulmonary involvement in systemic sclerosis: the detection of early changes by thin section CT scan, bronchoalveolar lavage and 99mTc-DTPA clearance. Respir Med 1989;83:403–14.
24. Beon M, Harley RA, Wessels A, Silver RM, Ludwicka-Bradley A. Myofibroblast induction and microvascular alteration in scleroderma lung fibrosis. Clin Exp Rheumatol 2004;22:733–42.
25. Pantin CF, Valind SO, Sweatman M, Lawrence R, Rhodes CG, Brudin L, et al. Measures of the inflammatory response in cryptogenic fibrosing alveolitis. Am Rev Respir Dis 1988;138:1234–41.
26. Hattori N, Degen JL, Sisson TH, Liu H, Moore BB, Pandrangi RG, et al. Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. J Clin Invest 2000;106:1341–50.
27. Plopis VA, Wilberding J, McLennan L, Liang Z, Cornelissen I, deFord ME, et al. A total fibrinogen deficiency is compatible with the development of pulmonary fibrosis in mice. Am J Pathol 2000;157:703–8.
28. Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A. Thrombin differentiates normal lung fibroblast to a myofibroblast phenotype via proteolytically activated receptor-1 and a

protein kinase C-dependent pathway. J Biol Chem 2001;276: 45184–92.

29. Bogatkevich GS, Ludwicka-Bradley A, Silver RM. Dabigatran, a direct thrombin inhibitor, demonstrates antifibrotic effects on lung fibroblasts. Arthritis Rheum 2009;60:3455–64.

30. Chambers RC, Dabbagh K, McAnulty RJ, Gray AJ, Blanc-Brude OP, Laurent GJ. Thrombin stimulates fibroblast procollagen production via proteolytic activation of protease-activated receptor 1. Biochem J 1998;333:121–27.