Evaluation of the effect of sildenafil on the microvascular blood flow in patients with systemic sclerosis: a randomised, double-blind, placebo-controlled study

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ABSTRACT

Objective. *To evaluate the effect of sildenafil as add-on therapy on the microvascular blood flow in patients with Raynaud's phenomenon (RP) secondary to systemic sclerosis (SSc).*

Methods. *In this double-blind, placebo-controlled study, 41 patients with RP secondary to SSc were randomly assigned to receive oral sildenafil 100 mg/day (21 patients, mean age 47.2 years) or placebo (20 patients, mean age 41.6 years) for 8 weeks. Patients were evaluated at baseline, 8 weeks after treatment, and 2 weeks after the end of the treatment. The primary outcome measures were the mean changes in finger blood flow (FBF) measured using laser Doppler imaging before and after cold stimulus at 8 weeks of treatment. Secondary endpoints included frequency and duration of RP attacks, Visual Analog Scale (VAS) score for RP severity, Raynaud's condition score, and serum levels of VEGF and endothelial progenitor cells (EPCs).*

Results. *After 8 weeks of treatment, the sildenafil group presented a significantly higher mean percentage change from baseline in FBF before cold stimulus (p=0.026), and in FBF after cold stimulus (p=0.028) compared with the placebo group. There was a significant improvement in the duration of RP and in the percentage change from baseline to week 8 in the RP VAS score in sildenafil compared with placebo. There were no changes in EPCs and VEGF levels after treatment in either group.*

Conclusions. *Sildenafil improved digital blood flow and RP symptoms in SSc patients after 8 weeks of treatment, and might be a good therapeutic option for secondary RP.*

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease characterised by microvascular damage and fibrosis of the skin and internal organs. Vascular abnormalities can be detected in the early stages of the disease and cause significant morbidity and mortality among SSc patients (1). Dysregulation of the vascular tone, of which Raynaud's phenomenon (RP) is the most frequent clinical manifestation, associated with endothelial dysfunction and intimal thickening of the vascular wall, leads to reduced blood flow and to chronic tissue ischaemia (2, 3).

Insufficient compensatory formation of new vessels (angiogenesis) and defective vasculogenesis characterised by altered numbers and function of bone marrow-derived endothelial progenitor cells (EPCs) might also contribute to the vascular abnormalities of SSc (3-5). The numbers of EPCs have been shown to be decreased in the early phases of the disease (6, 7), and their levels have been associated with disease severity and the presence of digital ulcers (8).

The treatment of RP in SSc patients remains challenging. Nitric oxide acts via the cyclic guanosine monophosphate (cGMP) pathway to mediate vasodilation and to inhibit cellular proliferation (9). Phosphodiestrase-5 (PDE-5) inhibitors work by inhibiting the action of the PDE-5 enzyme on the degradation of cyclic GMP, thus increasing the activity of nitric oxide and facilitating vasodilatation. PDE-5 inhibitors have been shown to inhibit platelet adhesion (10), to increase the circulating EPC levels in healthy men (11), and to improve endothelial function in diabetes patients (12). These drugs are approved for use in erectile dysfunction (13), in pulmonary arterial hypertension (14), and more recently, PDE-5 inhibitors have been used in the treatment of secondary RP in SSc and scleroderma-related diseases (15, 16). However, only

two randomised controlled trials evaluated the effect of sildenafil in patients with RP (17, 18). None of these studies evaluated the effect of sildenafil as add-on therapy in patients with RP.

This randomised, double-blind, placebo-controlled study, aimed to evaluate the effect of sildenafil as add-on therapy on the digital microvascular blood flow measured by laser Doppler imaging (LDI) in patients with RP secondary to SSc. In addition, we evaluated the effect of sildenafil on the clinical symptoms of RP, and on circulating EPCs and vascular endothelial growth factor (VEGF) levels in those patients.

Methods

Subjects

Patients with SSc meeting the 1980 American College of Rheumatology (ACR) classification criteria (19) or the 2001 LeRoy and Medsger criteria for early SSc (20) were consecutively recruited in the Rheumatology Division of the Federal University of São Paulo from August 2011 to November 2012. Patients were over 18 years old and should have at least one RP attack per day during the week before recruitment despite the use of other medications for RP. The patients who met the 1980 ACR classification criteria should have a disease duration of less than 5 years. Exclusion criteria were as follows: overlap with other connective tissue diseases, active smoking, diabetes, pregnancy, dyslipidaemia, liver disease, malignancies and current use of PDE-5 inhibitors or other drugs that might play a role in EPC mobilisation, such as statins and endothelin receptor antagonists.

Study design

This was a randomised, double-blinded, placebo-controlled 1:1 parallel treatment study.

The trial was registered at Clinicaltrials.gov (number: NCT01347008). This study was approved by the institutional review board of the Hospital São Paulo, São Paulo, Brazil and by the local ethics committee board (protocol number 1434/10). All patients provided their written informed consent to participate in the study before enrolment.

Each patient was evaluated at 4 visits: a screening visit (7 to 14 days before inclusion), a baseline visit (visit 1), 8 weeks after treatment (visit 2), and a follow-up visit 2 weeks after the end of treatment (visit 3). Clinical evaluation, laser Doppler imaging and blood sample collection were performed at visits 1, 2 and 3.

The primary outcome was changes in the finger blood flow (FBF) measured by LDI before and after a cold stimulus at 8 weeks of treatment. Secondary outcomes measures were the serum levels of EPCs, VEGF, frequency and duration of RP attacks, visual analog scale (VAS) score for RP severity, and the Raynaud's Condition Score. To evaluate a possible additional effect of sildenafil on the blood flow for a longer time, all parameters were also measured 2 weeks after the end of the treatment.

Randomisation

Patients were randomly assigned to receive 50 mg sildenafil twice daily (bid) (EMS Sigma Pharma, Ltd, Brazil) or identical placebo pills for 8 weeks. A pharmacist, who was not taking part in the study, performed simple randomisation using the GraphPad QuickCalcs. Active tablets and identical placebo tablets were encapsulated in identical bottles by the pharmacist, and sequentially numbered from 1 to 42 according to the randomisation list. All other concomitant medications remained unchanged during the study period.

Clinical assessment

At baseline, data regarding the disease features and global clinical evaluations, including information regarding RP duration, disease duration (defined as the onset of the first non-Raynaud's symptom), modified Rodnan Skin Score (mRSS) (21), the presence of digital ulcers, arthritis, positivities for anti-centromere antibodies (ACA) and antitopoisomerase I (anti-Scl-70) antibodies, interstitial lung disease, pulmonary arterial hypertension, and gastrointestinal involvement, were collected. The SSc patients were also classified into diffuse or limited cutaneous disease groups (22). Panoramic wide-field capillaroscopy was performed using a stereomicroscope, as previously described (23, 24). The scleroderma pattern was defined by the presence of enlarged and giant capillaries, loss of capillaries and microhaemorrhages (23). Drug therapy data were collected from all individuals.

RP symptoms were assessed using daily diary cards. Patients were instructed to record each RP event and the duration of the RP attack using the diary card during the week before the evaluation. At the end of each day, patients were instructed to complete the Raynaud's Condition Score (RCS), in which the difficulty the patient had had with RP in the last 24 hours should be estimated on a 0-10 scale (0 = no difficulty; 10 = extremely difficulty) (25). At each visit, the VAS score for RP severity from the scleroderma Health Assessment Questionnaire (sHAQ) was also recorded (26, 27).

Assessment of digital skin blood flow

Assessment of digital skin blood flow before and after cold stimulus (CS) was performed at visits 1, 2 and 3. Briefly, after acclimatisation for 60 minutes at a constant temperature of 24±1°C, the blood flow of the dorsum of four fingers (excluding the thumb) of the non-dominant hand was measured by laser Doppler imaging (Moor LDI-VR, Moor Instruments, Axminster, UK) before and after CS as previously described (28). All images were obtained at a beam scanning speed of 4 ms/pixel with a time of acquirement of 3 minutes and 15 seconds for each image. The distance between the photodetector and the examined surface was 40 cm. The blood flow of the dorsum of the four fingers was determined by establishing four regions of interests (ROI) at each finger defined as an area from the proximal interphalangeal joint up to and including the nailbed. The global mean finger blood flow (FBF) of the four fingers was derived (Moor LDI system software V5.2) and averaged. The blood flow was displayed in arbitrary perfusion units (PU). After baseline blood flow measurements, patients underwent a cold stimulus (submersion of both hands in water at 15°C for 1

minute). Further laser Doppler scanning and blood flow measurement was performed 20 minutes after CS.

Detection of EPCs and VEGF levels

For the detection of EPCs levels by flow cytometry, ten milliliters of venous blood was collected into an EDTA-containing tube in the morning and immediately transported to the laboratory for testing (29). Peripheral blood mononuclear cells were isolated using Ficoll density gradient centrifugation. One million cells were incubated with 4 μL of fluorescein-isothiocyanate (FITC)-conjugated CD34 (Southern Biotechnology Associates Inc., AL, USA), 4 μL of allophycocyanine (APC)-conjugated CD133 (Miltenyi Biotec, GE), and 8 μL of phycoerythrin (PE)-conjugated VEGF-R2 (R&D Systems, MN, USA). Polystyrene compensation beads conjugated with each of the fluorochromes were used to establish the positivity of each marker, and the fluorescence-minus-one technique was used to establish the cut-off value for each marker. Analyses of the labelled samples were performed using a FACSCanto II flow cytometer (Becton Dickinson Biosciences, CA, USA). At least 500,000 events were collected for each sample. The EPC population was identified as the CD34+/CD133+/VEGFR2+ cells within the lymphomononuclear (LMN) population. The data were analysed using FlowJo software v. 10.0.00003 (TreeStar, Inc., San Carlos, CA, USA). Plasma VEGF levels were assessed with enzyme-linked immunosorbent assays (R&D Systems, MN, USA) in accordance with the manufacturer's recommendations. The results are expressed as pg/mL.

Statistical analysis

A sample of 20 patients per treatment group was determined to yield 80% power at the 5% level (two-sided) to detect a mean difference change from baseline in FBF between the sildenafil and the placebo group of at least 3.0 PU, considering a standard deviation (SD) of 2.9 PU, based in the data of the pilot trial with sildenafil in RP (17).

Analysis was performed using the intention-to-treat principle. The Kolmogorov-Smirnov test was used to evaluate normality distribution of the data. Values are expressed as the mean ± SD or median ± interquartile range (IQR). Student's *t*-test was used for testing differences between the groups at baseline and for testing differences in the percentage change from baseline to V2 and V3 between the sildenafil and placebo groups. For abnormally distributed data, the Mann-Whitney test was used. Categorical variables were compared using Fisher's exact test or the chi-square test. For the other primary and secondary endpoints, analysis of covariance (ANCOVA) with treatment as a factor and baseline values as covariates was used. *P*-values less than 0.05 were considered significant. The statistical analysis was performed using SPSS statistical software for Windows v. 17.0 (SPSS Inc. Chicago, IL, USA).

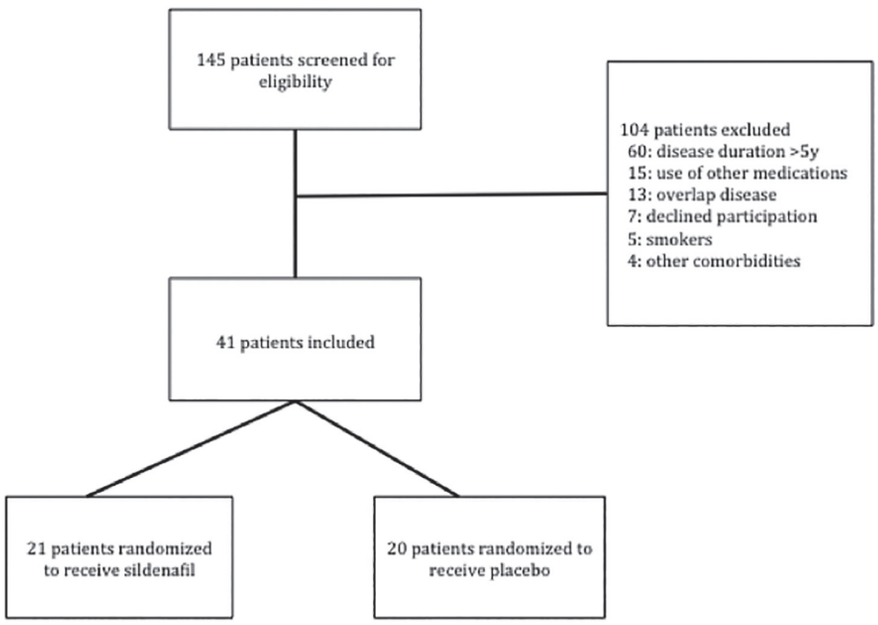

Fig. 1. Enrolment and disposition of the patients.

Results

Among the 145 patients submitted to the screening visit, 41 were included in the study. The most common reason for exclusion was disease duration >5 years. Twenty-one patients were randomly assigned to receive sildenafil, and twenty received placebo (Fig. 1). The demographic characteristics of the SSc patients are shown in Table I. All the participants were women. Fourteen patients had diffuse cutaneous SSc, 25 had limited cutaneous SSc, and two patients had early SSc according to the 2001 LeRoy criteria (one in the sildenafil and one in the placebo group). No patient was treated with prostanoids.

Laser Doppler imaging

The baseline FBF values before and after CS were slightly higher, although not significantly different, in the placebo group than in the sildenafil group (Table II). Therefore, the mean percentage change and the mean change from baseline were used to compare the two treatment groups. After 8 weeks of treatment, the sildenafil group presented a significantly higher mean percentage change from baseline in the FBF before CS (mean change of 28.1% in the sildenafil group *vs*. 1.7% in the placebo group, p=0.026), and in the FBF after CS compared with the placebo group (mean change of 62.4% *vs*. 6.8%, respectively, p=0.028) (Fig. 2). The percentage changes from baseline to visit 3 in the FBF values were similar between the sildenafil and placebo groups (mean change of 19.1% in the sildenafil group *vs*. -7.3% in the placebo group for FBF before CS, p=0.216; mean change of 38.1% in the sildenafil group *vs*. -4.7% in the placebo group for FBF 20 after CS, p=0.102).

As shown in Table II, there was a sig-

Table I. Baseline demographic and clinical characteristics of SSc patients.

	Sildenafil n=21	Placebo n=20	*p*
Age, years, mean ± SD	47.2 ± 10.9	41.6 ± 13.3	0.151
RP duration, years, mean ± SD	3.9 ± 2.5	3.4 ± 1.8	0.447
Disease duration, years, mean ± SD	3.4 ± 2.3	3.0 ± 1.8	0.505
Diffuse/Limited cutaneous SSc, n (%)	9/11 (43/28)	5/14 (25/50)	0.320
Modified Rodnan skin score, mean ± SD	7.4 ± 9.4	6.2 ± 8.9	0.689
Presence of digital ulcers, n (%)	3 (14.3)	1 (5)	0.606
Positive anti-Scl 70, n (%)	1 (5)	2 (10)	0.606
Positive ACA, n (%)	2 (9)	5 (25)	0.184
Frequency of scleroderma pattern on capillaroscopy, n (%)	19 (90.5)	16 (80)	0.410
PASP (mmHg) on echocardiography, mean ± SD	29.2 ± 5.0	28.4 ± 6.2	0.384
FVC (%) predicted on pulmonary function test, mean ± SD	83.9 ± 17.3	86.7 ± 14.3	0.880
Gastrointestinal involvement, n (%)	17 (81)	13 (65)	0.306
Arthritis, n (%)	7 (33)	11 (55)	0.215
Medications, n (%)			
Corticosteroids	5 (24)	10 (50)	0.111
Cyclophosphamide	2 (9)	4 (20)	0.410
Calcium channel blockers	17 (81)	12 (60)	0.181
ACE or ATB	6 (28.6)	8 (40)	0.520
Aspirin	4 (19)	1 (5)	0.343

ACA: anti-centromere antibodies; ACE: angiotensin converting enzyme inhibitor; ATB: angiotensin receptor blocker; FVC: forced vital capacity; PASP: pulmonary arterial systolic pressure; RP: Raynaud's phenomenon.

Table II. Finger blood flow (FBF) before and after cold stimulus (CS) in the sildenafil and placebo groups.

	FBF before CS		FBF after CS	
	Sildenafil	Placebo	Sildenafil	Placebo
Baseline	213.1 ± 87.9	241.9 ± 104.4	178.8 ± 92.4	218.6 ± 98.9
Week 8	260.0 ± 108.0	246.3 ± 122.6	257.7 ± 123.3	220.5 ± 119.9
Mean change from baseline to week 8 (95%CI)	44.58 (11.00,78.81)	4.43 (-51.25,50.12)	75.15 (18.58,120.41)	1.98 (-50.70,54.68)
Follow-up (V3)	228.9 ± 134.1	216.3 ± 117.1	221.1 ± 141.3	193.4 ± 113.8
Mean change from baseline to V3 (95%CI)	14.98 (-44.67,74.54)	-25.51 (-59.76,18.74)	40.23 (-19.37,89.84)	-25.2 (-59.76,18.74)

Values are expressed as the mean ± SD, unless otherwise stated. Comparison of FBF before and after CS between sildenafil and placebo at baseline: p=0.204, p=0.202, respectively. Differences of mean changes at week 8 compared with baseline between sildenafil and placebo groups, p=0.183 for FBF pre CS, p=0.046 for FBF after CS. Differences of mean changes on V3 compared with baseline between sildenafil and placebo groups, p=0.270 for FBF pre CS, p=0.09 for FBF after CS. CI: confidence interval.

nificant improvement in the FBF after CS in the sildenafil group compared with the placebo group after 8 weeks of treatment (p=0.046). The FBF values before CS presented a mean change of 44.58 PU in the sildenafil group compared with 4.43 PU in the placebo group after 8 weeks compared with baseline, although the comparison between groups was not significant. After the treatment (visit 3), the FBF values before and after CS in both groups were similar to the values at visit 1 (Table II).

Secondary end points

The clinical RP parameters were similar between the sildenafil and placebo groups at baseline. As shown in Table III, the duration of RP attacks in the last week significantly decreased after 8 weeks of treatment in the sildenafil group compared with the placebo group (p=0.042). The frequency of RP attacks, the RCS and the RP VAS were similar between the sildenafil group and the placebo group after 8 weeks (Table III). The percentage change from baseline to week 8 in the RP VAS score was significantly higher in the sildenafil group compared with the placebo group (mean percentage change of -35.9% versus -0.08%, respectively; p=0.014). There was also a trend towards improvement in the percentage change from baseline to week 8 in the duration of RP attacks in the sildenafil group compared with the placebo group (p=0.069). In the follow-up visit, there were no significant changes in any of the four clinical parameters evaluated between the two groups (Table III).

Four patients presented with active digital ulcers at the beginning of the study (3 in the sildenafil group and one in the placebo group). All of the 3 patients randomised to receive sildenafil experienced complete healing of the ulcers after the 8 weeks of treatment. At visit 3, all patients in the sildenafil group remained without digital ulcers. In the placebo group, the patient who presented with a digital ulcer remained with the ulcer throughout the study.

At the end of the 8 weeks of treatment and in the follow-up period, the EPC and VEGF levels did not show any significant differences between the groups (Table IV).

Adverse effects

Headache was more frequent among patients in the sildenafil group (7 patients, 33%) compared with the placebo group (01 patient, 5%) (p=0.022). Flushing (4 patients, 19%), and nausea (2 patients, 9%) were reported only in the sildenafil group. No patients withdrew from the study.

Discussion

In the present study, the use of sildenafil as add-on therapy for eight weeks was shown to increase the digital blood flow measured by LDI before and after cold stimulus in patients with RP secondary to SSc. There was also a significant improvement in the clinical symptoms of RP, as observed by a reduction in the mean duration of RP attacks and in the percentage change of the RP VAS score in the patients taking sildenafil.

RP secondary to SSc is more severe than idiopathic RP and is frequently associated with severe complications

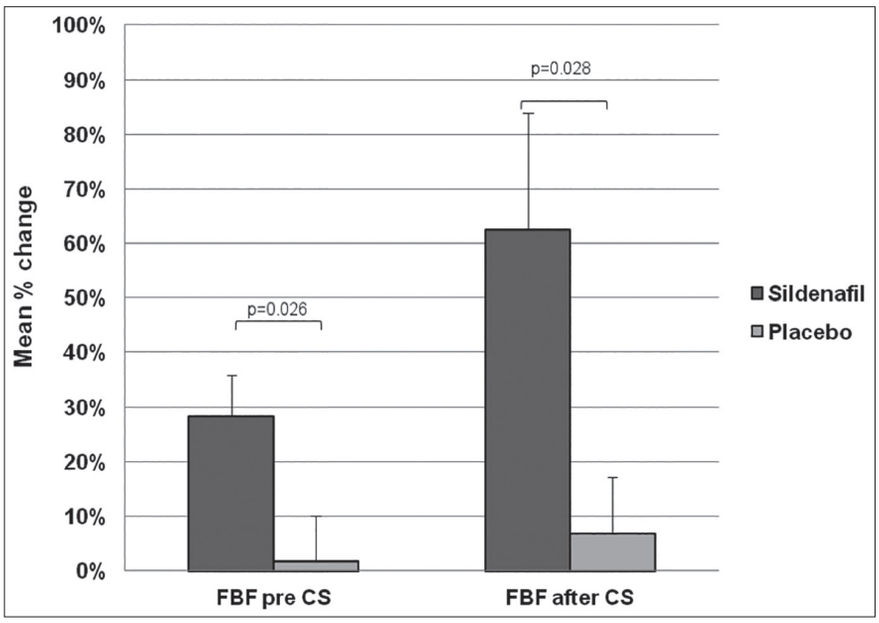

Fig. 2. Mean percentage change from baseline in FBF before and after CS.

such as digital ulcers and amputation (30). Several therapeutic options are available for the treatment of secondary RP, although these options have variable efficacy (31). In a recent meta-analysis evaluating the efficacy of PDE-5 inhibitors for secondary RP, a moderate reduction in the RCS, in the daily frequency of RP attacks, and in the daily duration of RP attacks was found (15). However, the studies included have some heterogeneity due to the inclusion of patients with other rheumatic diseases, the use of different dosages of medication and the evaluation of different PDE-5 inhibitors such as tadalafil and vardenafil.

To the best of our knowledge, this is the first study to use a laser Doppler imaging technique for the evaluation of sildenafil efficacy in patients with RP. The pioneer study of Fries et al. evaluated the capillary flow velocity using a laser Doppler anemometer (17). In contrast to the LDI, a laser Doppler anemometer allows the evaluation of microvascular flow velocity and provides only localised measurements in defined vessels. LDI has the advantages of being non-invasive and allowing the measurement of blood perfusion over a wide area of the skin, providing more reproducible results (28, 32). Although we used different methodology, our findings of an increase in the microvascular blood flow after treatment with sildenafil are in agreement with the results of Fries *et al*. (17), which observed a significant improvement in the microvascular flow velocity after sildenafil.

In our study, the microvascular blood flow was also evaluated after a cold stimulus. Interestingly, the increase in the blood flow was even more remarkable after CS, with a mean percentage change from baseline of 62.4% in the FBF in the sildenafil group. The cold challenge allows the evaluation of the functional response of the microcirculation after a vasoreactivity test (32). It is well known that patients with SSc present a lower blood flow at rest and a higher reactivity during cold with a delayed blood flow recovery (33, 34). PDE-5 inhibitors act by selectively in-

Table III. Clinical secondary end point results in the sildenafil and placebo groups.

	Daily frequency of RP attacks		Duration of RP attacks, min		RCS (0-10)		RP VAS (0-10)	
	Sildenafil (n=19)	Placebo (n=18)	Sildenafil (n=19)	Placebo (n=18)	Sildenafil (n=19)	Placebo (n=16)	Sildenafil (n=21)	Placebo (n=20)
Baseline (V1)	1.4 (3.3)	1.8 (2.7)	31.5 (46.5)	20.2 (47.6)	2.8 (4.2)	3.3 (3.2)	8.5 (9.5)	3.0 (9.0)
Week 8 (V2)	1.1 (2.5)	1.0 (3.0)	11.8 (21.0)	21.9 (22.6)	1.3 (3.2)	1.1 (5.6)	6.0 (8.25)	3.0 (9.0)
Mean % change from baseline to V2 (95%CI)	-10.8 (-52.83,31.16)	-19.6 (-55.39,16.17)	-39.1 (-63.90,14.39)	-1.2 (-37.64,35.12)	-11.7 (-49.60,36.18)	-40.8 (-74.6,7.34)	-35.9 (-63.58,-8.19)	-0.08 (-41.06,41.90)
Followup (V3)	1.4 (2.5)	1.4 (2.9)	11.2 (29.4)	20.0 (19.4)	3.1 (3.3)	1.8 (3.9)	6.0 (9.25)	5.0 (12.0)
Mean % change from baseline to V3 (95%CI)	11.5 (-53.51,63.41)	21.3 (-32.34,65.13)	-35.6 (-67.09,-4.16)	-0.36 (-33.90,33.18)	20.9 (-37.98,67.18)	-29.3 (-83.24,24.62)	24.1 (-39.98,78.15)	-0.06 (-39.17,52.07)

Values are expressed as the median (IQR), unless otherwise stated.
RP: Raynaud's phenomenon; RCS: Raynaud's Condition Score; RP VAS: visual analog scale score for RP severity.
V1 (sildenafil vs. placebo): daily frequency of RP attacks, p=0.615; mean duration of attacks, p=0.424; RCS, p=0.888; RP VAS, p=0.139.
V2 (sildenafil vs. placebo): daily frequency of RP attacks, p=0.744; mean duration of attacks, p=0.042; RCS, p=0.873; RP VAS, p=0.539.
V3 (sildenafil vs. placebo): daily frequency of RP attacks: p=0.484; mean duration of attacks, p=0.131; RCS, p=0.611; RP VAS p=0.976.
Mean percentage from baseline to week 8 (sildenafil vs. placebo): daily frequency of RP attacks, p=0.739; mean duration of attacks, p=0.069; RCS, p=0.316; RP VAS, p=0.014.
Mean percentage from baseline to visit 3 (sildenafil vs. placebo): daily frequency of RP attacks, p=0.822; mean duration of attacks, p=0.114; RCS, p=0.116; RP VAS, p=0.503.
CI: confidence interval.

Table IV. Endothelial progenitor cells (EPCs) and VEGF levels in the sildenafil and placebo groups.

	EPCs, number/10^6 lymphomononuclear cells		VEGF, pg/mL	
	Sildenafil (n=19)	Placebo (n=18)	Sildenafil (n=17)	Placebo (n=17)
Baseline (V1)	161.0 ± 103.7	170.7 ± 157.7	102.1 (171.3)	65.1 (82.1)
Week 8 (V2)	225.1 ± 163.90	235.5 ± 236.6	85.6 (332.5)	68.7 (188.2)
Followup (V3)	146.4 ± 123.2	135.6 ± 95.1	198.0 (487.9)	112.7 (323.7)

The data are presented as the mean ± SD for EPC levels and median (IQR) for VEGF.
V1: comparison of EPCs and VEGF between sildenafil and placebo: p=0.825, p=0.290, respectively.
V2: comparison of EPCs and VEGF between sildenafil and placebo: p=0.877, p=0.433, respectively.
V3: comparison of EPCs and VEGF between sildenafil and placebo: p=0.768, p=0.496, respectively.

hibiting the breaking down of cyclic GMP, a critical smooth muscle tone regulator (35). Therefore, the effect of sildenafil on the microvascular blood flow during a vasoreactivity test suggests that this medication could act by decreasing the vasoconstriction observed during RP attacks. In this context, sildenafil could be of most value in SSc patients by decreasing the severity of digital ischaemia during RP attacks.

The improvement in the clinical symptoms of RP observed in our study is consistent with previous reports (15). Daily diary cards associated with the RCS, SHAQ, RP VAS, and quality of life measures have been proposed as outcome measures in RP clinical trials (36, 37). There was a significant change in two clinical measures evaluated in our study: the mean duration of RP attacks and the severity of RP. Although the RCS and the frequency of RP attacks were shown to improve, there were no significant differences between the sildenafil and placebo groups. The RCS is a validated outcome measure that has been used in several clinical trials of RP (37-39). Nonetheless, previous studies have also reported significant changes in clinical parameters but not in the RCS (18), indicating that the clinical outcome measures might be not an accurate method of assessing the severity of the peripheral circulation in SSc patients (40). These findings reinforce the need for using objective methods, such as LDI, for the evaluation of RP and the microcirculation in therapeutic trials.

Digital ulcers are a severe complication of RP (41-43). It is estimated that about 60% of newly-diagnosed patients develop new digital ulcers within one year (41). Although the number of patients with active digital ulcers in our study was low, we observed complete healing of digital ulcers in the patients using sildenafil. Previous studies also reported the positive effect of PDE-5 inhibitors on digital ulcer healing (44, 45).

Changes in biomarker levels have been described and are potential therapeutic targets in SSc (46). EPCs play an important role in vascular homeostasis, actively contributing to vascular regeneration. In a previous study, we observed a decreased number of circulating EPCs in SSc patients with recent disease compared with controls (6). Moreover, previous studies reported increased levels of EPCs after PDE-5 inhibitors use (11, 47). However, in our study, there was no change in the number of circulating EPCs or in the levels of VEGF after treatment with sildenafil. The duration of the study and the dosage of sildenafil used might be not sufficient to modify these biomarkers. Moreover, it is possible that soluble markers are less sensitive to changes than blood flow and clinical measures among patients with SSc (18, 48).

Our study has several limitations, including the small sample size, due mostly to the exclusion of patients with no more than 5 years of disease. The fact that the patients included were from a tertiary centre might be also a potential source of selection bias for more severe disease. Moreover, patients were included over a long time period. The climate in our country is characterised by less temperature variation compared with Europe or North America. Nonetheless, we could not exclude an influence of outdoor temperature variation in the present results. The study was performed before the 2013 ACR/EULAR new classification criteria were published. Therefore, we used the 1980 ACR classification criteria for the diagnosis of SSc.

Several authors have highlighted the importance of the early diagnosis of SSc, aiming at the identification and treatment before irreversible vascular and fibrotic changes develop (49). As such, we decided to include patients with early disease and with disease duration of less than 5 years. The mean disease duration of the patients was of 3.2 years, indicating that the majority of the patients were in the early stages of SSc, when therapeutic interventions might be more successful.

Moreover, we decided to add sildenafil to the current treatment of the patients. Although there is no consensus, the addition of sildenafil to the first drug used for RP treatment has been recommended (50). In our study, calcium channel blockers were used in 70% of patients. Although these drugs are the first choice for RP treatment, adverse effects are common, and some patients do not tolerate their use. We could also observe that sildenafil, when used with other vasodilators, was safe and well tolerated and might be a worthwhile management option in the clinical practice. As expected, there was a loss of efficacy of sildenafil after the end of the treatment, suggesting the need for continuous or prolonged treatment.

Conclusions

Sildenafil as add-on therapy, at a dose of 100 mg/day for 8 weeks, was safe and well tolerated in patients with SSc. We showed, for the first time, a significant improvement in the digital microvascular blood flow evaluated using LDI in the sildenafil group compared with the placebo group. The present results also confirm the clinical efficacy of sildenafil for the treatment of RP observed in previous reports. These findings indicate that sildenafil could be considered a good treatment option for patients with RP secondary to SSc.

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