INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 23, no. 4, 1185-1193 (2010)

BOSENTAN TREATMENT FOR RAYNAUD'S PHENOMENON AND SKIN FIBROSIS IN PATIENTS WITH SYSTEMIC SCLEROSIS AND PULMONARY ARTERIAL HYPERTENSION: AN OPEN-LABEL, OBSERVATIONAL, RETROSPECTIVE STUDY

N. GIORDANO, L. PUCCETTI[1], P. PAPAKOSTAS, N. DI PIETRA[1], F. BRUNI[1], A.L. PASQUI[1], M. ACAMPA[1], V. BOCCHI[1], V. DONATI[1], M. VOGLINO[1], A. FIORAVANTI[2], A. MONTELLA, A. AUTERI[1], R. NUTI and M. PASTORELLI[1]

Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena; [1]Internal Medicine Unit, Department of Clinical Medicine and Immunological Sciences, University of Siena; [2]Rheumatology Unit, Department of Clinical Medicine and Immunological Sciences, University of Siena, Italy

Received June 6, 2010 - Accepted October 6, 2010

Raynaud's phenomenon (RP) and cutaneous fibrosis are the distinctive manifestations of scleroderma, in which Endothelin-1 plays a fundamental pathogenetic role. Bosentan, an Endothelin-1 receptor antagonist used for the treatment of pulmonary arterial hypertension, retards the beginning of new sclerodermic digital ulcers (DU). This open-label, observational, retrospective study verified the effect of Bosentan on RP and skin fibrosis in sclerodermic outpatients affected by pulmonary arterial hypertension without DU. Fourteen subjects (13 women, 1 man; mean age 60 ± 7.5 years; ten with limited and four with diffuse scleroderma) were observed at baseline (T0) and after four (T1), twelve (T2), twenty-four (T3) and forty-eight (T4) weeks during treatment with Bosentan. They were evaluated for daily quantity and duration of RP attacks and skin thickness (using modified Rodnan total skin score, MRSS). Videocapillaroscopic evaluation was performed at T0 and T4. Bosentan decreased significantly the number and duration of RP attacks, beginning at T2 ($p<0.05$). Videocapillaroscopy showed significant improvement of microcirculatory patterns at T4 ($p<0.05$). MRSS decreased throughout the study, reaching the statistical significance at T3 and T4 ($p<0.01$) in the whole cohort. The present data suggest that Bosentan is effective in stabilizing the microcirculation involvement and in improving skin fibrosis irrespective of scleroderma patterns.

Systemic sclerosis (SSc, also coined scleroderma) is a rare systemic disease of autoimmune origin and unknown aetiology, characterized by skin fibrosis, Raynaud's phenomenon (RP) and a variable involvement of the musculoskeletal, respiratory, cardiovascular, renal, and gastrointestinal system (1). More than 95% of patients suffering from SSc show RP, which often represents the first disease manifestation (2-4). Cutaneous fibrosis is the distinctive trait of scleroderma and, when affecting skin proximal to the metacarpophalangeal joints, is considered the major criterion for the classification of SSc (2). Histopathological abnormalities (e.g., vascular endothelial lesions, excessive collagen deposition, etc.) found in SSc are common in different organ tissues affected by the disease (5-6); moreover, skin fibrosis strongly correlates with the clinical course, internal organ involvement and

Key words: bosentan, Raynaud's phenomenon, skin fibrosis, systemic sclerosis, scleroderma, efficacy, tolerability

Mailing address: Prof. Nicola Giordano,
Department of Internal Medicine,
Endocrine-Metabolic Sciences and Biochemistry,
University of Siena, Hospital Santa Maria alle Scotte,
Viale Bracci 1, 53100, Siena, Italy
Tel: ++39 057 7233383 Fax: ++39 057 723446
e-mail: giordanon@unisi.it

0394-6320 (2010)

prognosis of scleroderma (7). Endothelial cell and capillary injury, collapse and obliteration by fibrosis are the main histological microvascular features (8). Endothelial cell dysfunction is a hallmark of SSc, even if the underlying pathophysiologic basis of the abnormal vascular responses is still unknown: it includes deregulation of vascular tone (9-10), increased capillary permeability (11-12) and high serum markers of endothelial cell injury (von Willebrand Factor, trombomodulin, soluble vascular adhesion cell molecules and others) (13-16). The endothelial dysfunction in SSc is further worsened by ischemia–reperfusion injury, through the release of cytokines, such as Tumor Necrosis Factor Alfa (TNFα), Interleukin-1 (IL-1), Interleukin-8 (IL-8), and Endothelin-1 (ET-1) and through the recruitment of platelets and neutrophils that generate superoxide radicals (17). ET-1 is a potent vasoconstrictor that plays a fundamental role in the pathogenesis of RP: increased blood levels of ET-1 were found in patients with primary and secondary RP, in particular RP associated to SSc (18-21). Enhanced expression of ET-1 has also been demonstrated in blood vessels, skin, lung, and kidney involved by scleroderma and increased ET-1 levels were found in SSc patients affected by diffuse disease with respect to the limited systemic form (22). *In vitro* studies suggest that ET-1 stimulates both lung fibroblasts to produce and contract extracellular matrix (ECM) and the capacity of Transforming Growth Factor beta (TGF-β) in inducing profibrotic genes (23-24). Furthermore ET-1 activates Intracellular Adhesion Molecule-1 (ICAM-1) expression on both normal and SSc fibroblasts (25) and interacts additively and cooperatively with Transforming Growth Factor beta (TGFβ) and Connective Tissue Growth Factor (CTGF, CCNN2) in inducing fibrosis (26). Of relevance ET-1-dependent vasoconstrictor activity is increased in SSc patients (27). So, ET-1 can be considered as a key mediator in this disease.

Bosentan, a dual (ETA and ETB) ET-1 receptor antagonist has been described to interact with several of the above-mentioned biological patterns (23-24). Bosentan is currently used for the treatment of patients with IPAH or associated to SSc (PAH-SSc) (28-29). Recently, Bosentan has also shown to be effective in the management of SSc digital ulcers (DU): in particular, this drug demonstrated efficacy in decreasing the number of new DU in SSc patients (30). While the clinical studies on Bosentan are substantial in SSc-associated DU, only few studies have investigated the potential activity of this drug on RP secondary to SSc with or without peripheral vascular ischemic lesions. In 2006, Selenko-Gebauer et al. (31), and subsequently Hettema et al. (32), reported that Bosentan reduces the impact of Raynaud's disease, evaluated by clinical parameters. In particular, in 2007, Hettema et al. asserted that Bosentan treatment results in an encouraging improvement in frequency, duration and severity of RP attacks in SSc patients, but without a demonstrated objective improvement in blood flow (32). Sfikakis et al. affirmed that Bosentan improved endothelial function without affecting hemodynamic parameters or endothelial activation-related processes, thus supporting a direct, reversible effect of ET-1 in SSc-associated vascular injury (33). Other authors have demonstrated that Bosentan could improve not only RP but also cutaneous fibrosis in SSc patients (34-36).

However, contrasting data have been reported; Bosentan did not induce significant changes in vasodilator responses, capillary permeability, capillary density and vascular healing (37). Recently, Nguyen et al. showed that in seventeen SSc subjects treated with the drug for four weeks, Bosentan was not effective in SSc-related RP without pre-existing digital ulcers, but that it might benefit functional impairment in these patients (38). Moreover, Kuhn et al. demonstrated that Bosentan was effective in reducing skin fibrosis in ten SSc patients treated with the drug for twenty weeks (39). On the basis of these controversial opinions, we performed an open-label, observational, retrospective, study on patients affected by PAH-SSc who were on Bosentan treatment, testing the potential activity of the drug on RP without DU, and skin fibrosis, both distinctive manifestations of scleroderma. In particular, the primary aim of the study is the evaluation of RP during the one-year treatment period. The secondary aim includes cutaneous fibrosis evaluation and the assessment of safety and tolerability of Bosentan.

MATERIALS AND METHODS

Fourteen SSc outpatients with RP, affected by dSSc or lSSc, as defined by LeRoy et al. (2) who met the preliminary American College of Rheumatology (formerly

the American Rheumatism Association) classification criteria for SSc, were included in the study. All patients were consecutively recruited in the Department of Internal Medicine of the University of Siena (from October 2007 to June 2009). Each subject gave fully informed voluntary written consent. The study protocol followed the principles of the Declaration of Helsinki and was approved by the ethic committee of the Recruiting Center. All patients had a diagnosis of SSc-PAH, suspected by clinical signs and symptoms and confirmed by pulmonary function tests, exercise capacity test (6-min walk test) and transthoracic echocardiodoppler for the evaluation of pulmonary artery systolic pressure (PASP) (n.v. ≤35 mmHg) as previously described in details (40). No patient underwent right heart catheterization because transthoracic echocardiodoppler is able to accurately classify patients with chronic heart failure with or without PAH (40), avoiding subjecting the compromised patients to an invasive, potentially dangerous procedure. The patients continued with prior medications, including oral vasodilating drugs, ACE inhibitors, d-penicillamine and corticosteroids (≤10mg of prednisone or equivalent per day). All the above-mentioned drugs had been started more than twelve months before the beginning of the study, without changes during the observational period. No treatment with parenteral prostanoids within the previous six months was allowed and no patient had received previous treatment with Bosentan. Patients with active renal crisis (abrupt onset of malignant hypertension and rapidly progressive renal failure), advanced life-threatening cardiopulmonary disease, severe liver disease and severe anemia (hemoglobin concentration ≤ 8 gr/dl) were excluded. Other exclusion criteria were pregnancy and current breast-feeding. The patients received Bosentan 62.5 mg twice daily for four weeks and then 125 mg twice daily for more than one year (ranging from forty-nine to seventy-two weeks, 60±9.2 weeks). Blood chemistry, including liver function and total blood count, was monitored every fourteen days during the first four weeks followed by every four weeks until twenty-four weeks, and subsequently at forty-eight weeks by standard methods. Antinuclear antibodies (ANAs) by indirect immunofluorescence assay (Immuno Concepts) and Antibodies to extractable nuclear antigens (ENAs) (FEIA, Phadia) were evaluated at baseline, twenty-four and forty-eight weeks. The patients were evaluated for PAH-SSc by clinical aspects, pulmonary function and exercise capacity (6-min walk test) tests, and echo-cardiographic parameters at baseline (T0) and after four (T1), twelve (T2), twenty-four (T3) and forty-eight (T4) weeks. At T0 the presence or absence of RP per day and the duration of attacks were investigated; subsequently (T1, T2, T3, T4), the patients had to keep a diary reporting the presence or absence of RP per day and the duration of attacks. All patients underwent nailfold videocapillaroscopy (VCP) at T0 and T4 because of its utility in systemic diseases (41).

VCP represents the most accurate technique to identify the microvascular "scleroderma pattern", to evaluate disease progression over time and responsiveness to vasoactive drugs (42-43). In this study a VCP of last generation Video Cap, MDS group, software release 8,9 was used. The images were taken by a videoprobe connected to the instrument by an optical fiber cable which allows a 200x magnification, as previously described (44). The patients were examined in the same environmental conditions in a temperature-controlled laboratory (21°C to 24°C). If necessary, room temperature was adjusted by using fan heaters or air conditioning. Qualitative parameters such as visibility, dystrophies, sludging, capillary density alterations, numeric abnormalities, avascularised zones, megacapillary presence, haemorrhages, oedema, background pallor, capillary comb alteration, saw-toothed aspect of microvessel and wall incisures, were evaluated in the whole cohort. A modified version of the cited semi-quantitative rating scales proposed for systemic sclerosis was used in scoring microvascular changes in our patients: in particular, a semi-quantitative scale was performed (as 0=normal, 1=slight, 2=mild, 3=severe) to quantify all described parameters (41). The enrolled patients were tested by cold, as reported in the international literature (45). Moreover, at T0, T1, T2, T3 and T4, skin thickness was assessed using the modified Rodnan total skin score (MRSS) that represents the best clinical evaluation of dermal thickness and is both accurate and reliable (46). Skin thickening was assessed by palpation of the skin in seventeen areas of the body (fingers, hands, forearms, arms, feet, legs and thighs, face, chest and abdomen) using a 0–3 scale, where 0=normal, 1=mild thickness, 2=moderate thickness, and 3=severe thickness. Total skin score can range from 0 (no thickening) to 51 (severe thickening in all 17 areas). We underline that the clinical and instrumental (especially MRSS) evaluations were performed by the same physician. Percentages and means ± sd were used to describe the cohort's demographics.

In order to evaluate the differences at different time-points between each measured parameter, a statistical analysis employing ANOVA for repeated measures and correction by Bonferroni-Dunn method was applied to the cohort of studied subjects. Baseline and in-study values of categorized variables were evaluated by the Mann-Whitney U test for differences among groups. A $p < 0.05$ was accepted as statistically significant. Each calculation was made employing the SPSS version 16.0 package.

RESULTS

Demographic and clinical data of the studied

population are shown in Table I. Only one patient was male, aged 59 years and affected by lSSc for about 74 months. All fourteen patients completed the study. Bosentan was well tolerated from clinical and laboratory standpoints. Only one patient showed increased serum liver enzyme levels after three months of treatment, but Bosentan reduction to 62.5 mg b.i.d. allowed normalization within 1 month. Bosentan treatment resulted in a significant reduction in number and daily duration of RP attacks, as shown in Table II. Briefly, the number of RP attacks per day was 3.4±1.8 in lSSc patients and 3.8±1.7 in dSSc patients at baseline, without a statistical difference between groups. The number of RP attacks per day started to decrease at T1, even if the decrease reached statistical significance at T2 ($p<0.05$) and remained stable at T3 and T4. The same outcome was highlighted in both lSSc and dSSc patients, without a statistical difference between groups. Duration (min) of RP attacks per day was 62.5±43.7 in lSSc patients and 61.0±38.9 in dSSc patients at baseline, without a statistical difference between groups. Similarly, the duration of RP attacks per day decreased at T1, reached statistical significance at T2 ($p<0.05$) and remained stable at T3 and T4. The same outcome was highlighted again in lSSc and dSSc patients, without a statistical difference between the two groups. Each qualitative parameter, evaluated by nailfold VCP, ranged from 0.22 to 1.42 score in a 0–3 scale at T0, depending on slight-mild changes of microvasculature (Fig. 1). Nailfold VCP (at least six recording for each patient for each scenario) showed that visibility and sludging improved significantly at T4 ($p<0.05$); capillary density alteration, avascularised zone, oedema and background pallor also improved, even if they did not reach statistical significance; the remaining examined parameters remained stable (Fig. 1). Qualitative parameters were evaluated in all patients, without differentiating lSSc patients and dSSc ones, because of no statistical power between groups. The MRSS was 18.5±7.3 in lSSc patients and 23.0±6.3 in dSSc at baseline, without a statistical difference between the groups. The average MRSS reduced during Bosentan treatment: similar patterns of improvements in the MRSS were found in both the lSSc and dSSc patients' groups; the improvement reached statistical significance at T3 ($p<0.05$) and remained stable at T4 (Fig. 2). In particular, MRSS decreased by 45.95% in lSSc patients and 37.39% in dSSc patients, from T0 to T4. The evaluation of ANAs and ENAs, at T0, T3 and T4, did not change significantly in pattern and title in lSSc patients nor in dSSc ones. Even if the aims of our study are to assess the effect of Bosentan on SSc RP and skin fibrosis, for the sake of completeness we briefly describe the outcome of PASP and change of functional and therapeutic class [New York Heart Association (NYHA) classification] (47). At baseline, PASP (mmHg, n.v.≤35), was 50.07±3.5 and decreased progressively, reaching statistical significance at T2 (39.07±2.6), at T3 (35.61±3.2) and at T4 (34.92±2.3) ($p<0.01$). Regarding NYHA classification, a significant improvement was shown of the NYHA classes in all the patients. In particular, at T0 no patient was in NYHA class I, 4 patients were in NYHA class II (28.6%), 9 patients in NYHA class III (64.3%) and 1 patient in NYHA class IV (7.1%). At T1, 1 patient was in NYHA class I (7.1%), 5 patients in class II (35.8%), 7 in class III (50%) and 1 in class IV (7.1%). At T2, 4 patients were in class I (28.6%), 6 in class II (42.8%), 4 in class III (28.6%) and none in class IV. At T3 and T4, 6 patients were in class I (42.8%), 6 in class II (42.8%), 2 in class III (14.4%) and none in class IV.

DISCUSSION

The present open-label, observational, retrospective study shows that Bosentan is able to reduce the number and duration of RP attacks per day in SSc patients without DU. The evidence

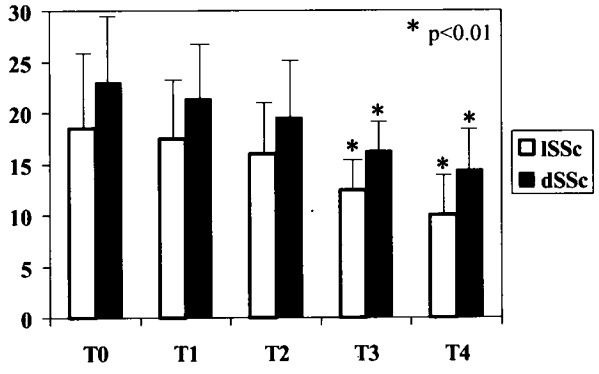

Fig. 1. *VCP qualitative score for the whole cohort of SSc patients before and after Bosentan treatment. Mann-Whitney U test, * $p<0.05$.*

of this activity on microcirculation was confirmed by the data obtained by VCP; we can therefore affirm that Bosentan treatment was able to stabilize microvasculature in all the treated patients, so that no patient worsened. We believe that these last findings are particularly interesting because, to the best of our knowledge, they have not been previously reported and they add data to the body of evidence concerning positive vascular effects of Bosentan despite some contrasting reports, as previously described (37-39).

However, the impact of Bosentan on SSc has been evaluated by other authors mainly in terms of its effect on DU, mostly because they are the major clinical problem in SSc, occurring in about one-third of patients, causing local pain and functional impairment and having a negative effect on the quality of life for patients with SSc (48). In this setting, RAPIDS-2 was a large scale trial, including one-hundred-eighty-eight SSc patients with at least one digital ulcer enrolled from forty-one different referral centers in USA and Europe (50). The study failed to detect an impact of Bosentan on the healing of ulcers, but confirmed the capability to prevent the development of new digital ulcers, inducing an improvement in hand functionality and reducing pain (49).

A further relevant difference between the present and other studies is the longer duration of treatment lasting more than 1 year compared to fourteen or sixteen weeks. Such a relevant difference could influence our findings because of increased exposure to the drug without induction of tolerance. Moreover, the long period of therapy assured the observation of the patients in every season, and RP resulted distributed across different periods for each subject without a clear relation to climatic changes.

Previous data reported positive effects of Bosentan in dermal sclerosis (34-36, 39); however, limited evidence dealing with the limited or diffuse form of SSc was available (36). Furthermore, MRSS changes could be related to the natural evolution of skin involvement in SSC, and the absence of a placebo group cannot exclude such an option in the present results of improved MRSS. However, we evaluated MRSS in order to verify putative differences in Bosentan action according to the forms of SSc, despite the small sample size, and we found a similar improvement.

Such findings agree with previous observations that Bosentan improves skin fibrosis (36-39). The latter could be relevant because of the increased number of evaluated subjects in different studies and could suggest a new potential therapeutic approach to SSc, the improvement of skin fibrosis at the present time being observed mainly in patients treated with immunosuppressants (metothrexate, cyclophosphamide, cyclosporin A, chlorambucil),

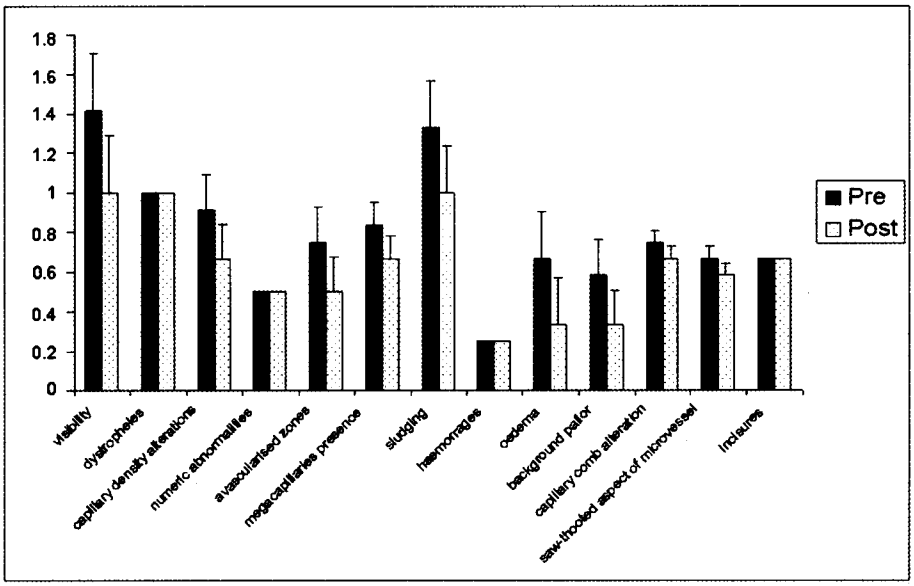

Fig. 2. *Outcome of Modified Rodnan Total Skin score for lSSc and dSSc patients during Bosentan treatment. ANOVA for repeated measures and Bonferroni-Dunn. * $p<0.01$ compared with baseline*

Table I. *Demographic and clinical data of SSc patients (N.14).*

All patients, No.	14			
Age, m±sd, yrs	60±7.5			
Duration of SSc, m±sd, yrs	6±2.6			
	lSSc		dSSc	
	No.	%	No.	%
Male	1	10%	0	0
Female	9	90%	4	100%
Ethnicity Caucasian	10	100%	4	100%
Type of SSc	10	71.43%	4	28.57%
Clinical characteristics				
Raynaud Phenomenon	10	100%	4	100%
Sclerodactyly	10	100%	4	100%
Teleangectasia	7	70%	3	75%
Calcinosis	4	40%	1	25%
Esophageal dismotility	6	60%	3	75%
Interstitial fibrosis lung disease	1	10%	2	50%
Chest and/or limb skin fibrosis	0	0	4	100%
Oliguric renal failure	0	0	1	25%
Tendon friction rubs	0	0	1	25%
Laboratory				
Anti-centromere antibodies	5	50%	1	25%
Anti-DNA topoisomerase antibodies	2	20%	2	50%
Anti-RNA polimerasi antibodies	2	20%	1	25%
Treatment				
Penicillamine	10	100%	4	100%
6-methylprednisolone (≤10 mg/die)	1	10%	2	50%
Calcium channel blockers	10	100%	4	100%
Metoclopramide	6	60%	3	75%
ACE-inhibitors	0	0	3	75%

Table II. *Outcome data of the Raynaud's phenomenon (RP) diary (§) in lSSc (no 4) and dSSc (N. 10) patients (pts) at baseline and during Bosentan treatment.*

	T0	T1	T2	T3	T4
N. RP attacks/day lSSc	3.4±1.8	3.1±2.1	2.1±1.3*	2.0±1.1*	2.1±1.4*
N. RP attacks/day dSSc	3.8±1.7	3.3±1.8	2.4±1.9*	2.3±1.2*	2.1±1.8*
Duration (min) Rp attacks/day lSSc	62.5±43.7	58.3±36.5	32.0±9.1**	29.0±12.3**	22.1±13.8**
Duration (min) Rp attacks/day dSSc	61.0±38.9	57.3±35.0	30.0±10.5**	31.1±11.3**	29.2±13.7**

RP: Raynaud's phenomenon
Data are presented as mean±sd, ANOVA for repeated measures and Bonferroni-Dunn method,
** $p<0.05$ vs baseline, ** $p< 0.01$ vs baseline*

d-Penicillamine and prostacyclins in various combinations especially in "early" dSSc (51-54). Other most recent treatments targeting fibrosis, such as interferons, seem to exert marginal effects on skin involvement in SSc (54), while tyrosine kinase inhibitors and recombinant human anti-TGF beta 1 antibody manifested anti-fibrotic effects in vitro and in vivo both in animal studies and in a limited number of unselected cases refractory to other treatments, thus requiring further evidence (54).

In conclusion, our data suggest that Bosentan is able to improve microcirculation and skin fibrosis in SSc patients, probably reducing ET-1 activity in inflammation, tissue remodeling and fibrosis, as previously stated. Further evidence should be obtained to identify an assessment on any skin histological changes to be used to objectively evaluate the drug action on skin fibrosis. Longer-term, larger, ad hoc, studies are warranted to update the above-reported body of evidence in order to confirm a putative role for Bosentan in SSc control.

Limitations of the study

The major limitation of this study is the retrospective design, which does not include a control group and a small sample size. However, the setting of studied subjects did not allow ethically a control group treated with placebo. Furthermore, the small sample size was compatible with the rarity of the condition.

ACKNOWLEDGEMENTS

This work was partially supported by a grant from the University of Siena to N.G. and M.P.

REFERENCES

1. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. NEJM 2009; 360:1989-20.
2. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, Rowell N, Wollheim F. Scleroderma (systemic sclerosis) – Classification, subsets and pathogenesis. J Rheumatol 2002; 15: 202-5.
3. Wigley FM. Clinical practice. Raynaud's phenomenon. N Engl J Med 2002; 347:1001-8.
4. Steen V, Denton CP, Pope JE, Matucci-Cerinic M. Digital ulcers: overt vascular disease in systemic sclerosis. Rheumatology 2009; 48:19-24.
5. Derk CT, Jimenez SA. Systemic sclerosis: current views of its pathogenesis. Autoimmun Rev 2003; 2: 181-91.
6. Varga J. Systemic sclerosis: an update. Bull NYU Hosp Jt Dis 2008; 66:198-202.
7. Krieg T, Takehara K. Skin disease: a cardinal feature of systemic sclerosis. Rheumatology 2009; 48:14-8.
8. Fleming JN, Nash RA, Mahoney WM Jr, Schwartz SM. Is scleroderma a vasculopathy ? Curr Rheumatol Rep 2009; 11:103-10.
9. Kahaleh B, Matucci-Cerinic M. Raynaud's phenomenon and scleroderma. Dysregulated neuroendothelial control of vascular tone. Arthritis Rheum 1995; 38:1-4.
10. Anderson ME, Campbell F, Hollis S, Moore T, Jayson MI, Herrick AL. Non-invasive assessment of digital vascular reactivity in patients with primary Raynaud's phenomenon and systemic sclerosis. Clin Exp Rheumatol 1999; 17:49-54.
11. Bollinger A, Jager K, Siegenthaler W. Microangiopathy of progressive systemic sclerosis. Evaluation by dynamic fluorescence videomicroscopy. Arch Intern Med 1986; 146:1541-5.
12. Grassi W, Core P, Carlino G, Cervini C. Effects of peripheral cold exposure on microvascular dynamics in systemic sclerosis. Arthritis Rheum 1994; 37:384-90.
13. Gruschwitz MS, Hornstein OP, von Den DP. Correlation of soluble adhesion molecules in the peripheral blood of scleroderma patients with their in situ expression and with disease activity. Arthritis Rheum 1995; 38:184-9.
14. Herrick AL, Illingworth K, Blann A, Hay CR, Hollis S, Jayson MI. Von Willebrand factor, thrombomodulin, thromboxane, beta-thromboglobulin and markers of fibrinolysis in primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis 1996; 55:122-7.
15. Andersen GN, Caidahl K, Kazzam E, Petersson AS, Waldenstrom A, Mincheva-Nilsson L, Rantapaa-Dahlqvist S. Correlation between increased nitric oxide production and markers of endothelial activation in systemic sclerosis: findings with the soluble adhesion molecules E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion

molecule 1. Arthritis Rheumatism 2000; 43:1085-93.
16. Cerinic MM, Valentini G, Sorano GG, et al. Blood coagulation, fibrinolysis, and markers of endothelial dysfunction in systemic sclerosis. Semin Arthritis Rheum 2003; 32:285-95.
17. Wigley FM. Vascular disease in scleroderma. Clin Rev Allergy Immunol 2009; 36:150-75.
18. Yamane K, Kashiwagi H, Suzuki N, Miyauchi T, Yanagisawa M, Goto K, Masaki T. Elevated plasma levels of endothelin-1 in systemic sclerosis. Arthritis Rheum 1991; 34:243-4.
19. Kahaleh MB. Endothelin, an endothelial-dependent vasoconstrictor in scleroderma – enhanced production and profibrotic action. Arthritis Rheum 1991; 34:978-83.
20. Vancheeswaran R, Magoulas T, Efrat G, Wheeler-Jones C, Olsen I, Penny R, Black CM. Circulating endothelin-1 levels in systemic sclerosis subsets--a marker of fibrosis or vascular dysfunction? J Rheumatol 1994; 21:1838-44.
21. Morelli S, Ferri C, Polettini E, Bellini C, Gualdi GF, Pittoni V, Valesini G, Santucci A. Plasma endothelin-1 levels, pulmonary hypertension, and lung fibrosis in patients with systemic sclerosis. Am J Med 1995; 99:255-60.
22. Kuryliszyn-Moskal A, Klimiuk PA, Sierakowski S. Soluble adhesion molecules (sVCAM-1, sE-selectin), vascular endothelial growth factor (VEGF) and endothelin-1 in patients with systemic sclerosis: relationship to organ systemic involvement. Clin Rheumatol 2005; 24:111-6.
23. Shi-Wen X, Chen Y, Denton CP, et al. Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. Mol Biol Cell 2004; 15:2707-19.
24. Shi-wen X, Kennedy L, Renzoni EA, Bou-Gharios G, du Bois RM, Black CM, Denton CP, Abraham DJ, Leask A. Endothelin is a downstream mediator of profibrotic responses to transforming growth factor beta in human lung fibroblasts. Arthritis Rheum 2007; 56:4189-94.
25. Leask A. Signaling in fibrosis: Targeting the TGF beta, endothelin-1 and CCN2 axis in scleroderma. Front Biosci 2009; 11:115-22.
26. Shiwen X, Leask A, Abraham DJ, Fonseca C. Endothelin receptor selectivity: evidence from in vitro and pre-clinical models of scleroderma. Eur J Clin Invest 2009; 39:19-26.
27. Cardillo C, Schinzari F, Melina D, Mores N, Borsello S, Peluso G, Zoli A, Ferraccioli G. Improved endothelial function after endothelin receptor blockade in patients with systemic sclerosis. Arthritis Rheum 2009; 60:1840-44.
28. Steiner MK, Preston IR. Optimising endothelin receptor antagonist use in the management of pulmonary arterial hypertension. Vasc Health Risk Manag 2008; 4:943-52.
29. McLaughlin V, Humbert M, Coghlan G, Nash P, Steen V. Pulmonary arterial hypertension: the most devastating vascular complication of systemic sclerosis. Rheumatology 2009; 48:25-31.
30. Tsifetaki N, Botzoris V, Alamanos Y, Argyriou E, Zioga A, Drosos AA. Bosentan for digital ulcers in patients with systemic sclerosis: a prospective 3-year follow-up study. J Rheumatol 2009; 36:1550-1.
31. Selenko-Gebauer N, Duschek N, Minimair G, Stingl G, Karlhofer F. Successful treatment of patients with severe secondary Raynaud's phenomenon with the endothelin receptor antagonist bosentan. Rheumatology 2006; 45:45-8.
32. Hettema ME, Zhang D, Bootsma H, Kallenberg CG. Bosentan therapy for patients with severe Raynaud's phenomenon in systemic sclerosis. Ann Rheum Dis 2007; 66:1398-9.
33. Sfikakis PP, Papamichael C, Stamatelopoulos KS, Tousoulis D, Fragiadaki KG, Katsichti P, Stefamadis C, Mavrikakis M. Improvement of vascular endothelial function using the oral endothelin receptor antagonist Bosentan in patients with systemic sclerosis. Arthritis and Rheumatism 2007; 56:1985-93.
34. Humbert M, Cabane J. Successful treatment of systemic sclerosis digital ulcers and pulmonary arterial hypertension with endothelin receptor antagonist bosentan. Rheumatology 2003; 42:191-3.
35. Alegre-Sancho JJ, Roman-Ivorra JA, Fernandez-Carballido C. Systemic sclerosis: improvements of cutaneous fibrosis on bosentan therapy. Ann Rheum Dis 2004; 63:339.
36. Funauchi M, Kishimoto K, Shimazu H, Nagare Y,

Hino S, Yano T, Kinoshita K. Effects of bosentan on the skin lesions: an observational study from a single center in Japan. Rheumatol Int 2009; 29:769-75.

37. Hettema ME, Zhang D, Stienstra Y, Smit AJ, Bootsma H, Kallenberg CG. No effects of Bosentan on microvasculature in patients with limited cutaneous systemic sclerosis. Clin Rheumatol 2009; 28:825-33.
38. Nguyen VA, Eisendle K, Gruber I, Hugl B, Reider D, Reider N. Effect of the dual endothelin receptor bosentan on Raynaud's phenomenon secondary to systemic sclerosis: a double-blind prospective, randomized, placebo-controlled pilot study. Rheumatology 2010; 49:583-7.
39. Kuhn A, Haust M, Ruland V, Weber R, Verde P, Felder G, Ohmann C, Gensch K, Ruzicka T. Effect of bosentan on skin fibrosis in patients with systemic sclerosis: a prospective, open-label, non-comparative trial. Rheumatology 2010; 49:1336-45.
40. Kowal-Bielecka O, Avouac J, Pittrow D, et al. EPOSS Group. Echocardiography as an outcome measure in scleroderma-related pulmonary arterial hypertension: a systematic literature analysis by the EPOSS group. J Rheumatol 2010; 37:105-15.
41. Jouanny P, Scmidt C, Feldman L, Scmitt J. Peri-ungual capillaroscopy. Value in the diagnosis of systemic diseases. Press Med 1993; 22:1256-60.
42. Cutolo M, Grassi W, Matucci Cerinic M. Raynaud's phenomenon and the role of capillaroscopy. Arthritis Rheum 2003; 48:3023-30.
43. Maricq HR, Valter I. A working classification of scleroderma spectrum disorders: a proposal and the results of testing on a sample of patients. Clin Exp Rheumatol 2004; 22:5-13.
44. Pastorelli M, Pasqui AL, Puccetti L, et al. Quantitative evaluation of microvessels in Behçet's disease. Adv Exp Med Biol 2003; 528:427-33.
45. Foerster J, Kuerth A, Niederstrasser E, Krautwald E, Pauli R, Paulat R, Eweleit M, Piemekasten G, Worm M. A cold-response index for the assessment of Raynaud's phenomenon. J Dermatol Sci 2007; 45: 113-20.
46. Clements P, Lachenbruch P, Seibold JR, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995; 22:1281-5.
47. Bennett JA, Riegel B, Bittner V, Nichols J. Validity and reliability of the NYHA classes for measuring research outcomes in patients with cardiac disease. Heart Lung 2002; 31:262-70.
48. Ramirez A, Varga J. Pulmonary hypertension in systemic sclerosis: clinical manifestations, pathophysiology, evaluation, and management. Treat Respir Med 2004; 3:339-52.
49. Launay D, Humbert M, Hachulla E. Pulmonary arterial hypertension and systemic sclerosis. Press Med 2006; 35:1929-37.
50. Seibold JR, Denton CP, Furst DE. Bosentan prevents occurence but does not speed healing of digital ulcers in patients with systemic sclerosis. ACR; San Diego, USA 2005.
51. Zandman-Goddard G, Tweezer-Zaks N, Shoenfeld Y. New therapeutic strategies for systemic sclerosis-a critical analysis of the literature. Clin Dev Immunol 2005; 12:165-73.
52. Derk CT, Huaman G, Jimenez SA. A retrospective randomly selected cohort study of D-penicillamine treatment in rapidly progressive diffuse cutaneous systemic sclerosis of recent onset. Br J Dermatol 2008; 158:1063-8.
53. Scorza R, Caronni M, Mascagni B, et al. Effects of long-term cyclic iloprost therapy in systemic sclerosis with Raynaud's phenomenon. A randomised, controlled study. Clin Exp Rheumatol 2001; 19:503-8.
54. Bournia VK, Vlachoyiannopoulos P, Selmi C, Moutsopoulos H, Gershwin ME. Recent advances in the treatment of Systemic Sclerosis. Clinic Rev Allerg Immunol 2009; 36:176-200.