A Randomized, Blinded, Parallel Group, Placebo Controlled Pilot Study Evaluating the Effect of PVAC Treatment in Patients with Diffuse Systemic Sclerosis

MARK C. GENOVESE, ELIZA F. CHAKRAVARTY, DAVID L. BOYLE, ZUHRE TUTUNCU, CHRISTINE M. THORBURN, MERITA HALILHODZIC, STEWART KROLL, JAN BAUGHMAN, STANFORD STEWART, and ARTHUR KAVANAUGH

ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is a disorder characterized by progressive thickening of the skin; there is no effective therapy. PVAC, a potential therapeutic agent derived from delipidated, deglycolipidated *Mycobacterium vaccae*, has shown effects on cutaneous disease in animal models of SSc. We evaluated the safety and possible biologic effect of intradermal injections of PVAC in patients with diffuse SSc.

Methods. Eighteen patients enrolled in this double blind, placebo controlled, randomized, 24 week pilot study. All patients met criteria for diffuse SSc without evidence of significant renal dysfunction, pulmonary fibrosis, pulmonary hypertension, or congestive heart failure. Patients received 8 intradermal injections of 15 μg PVAC, 50 μg PVAC, or placebo at 3 week intervals. The primary efficacy endpoint was the change in Modified Rodnan Skin Score (MRSS) at Week 24. Each of the active drug arms was compared to placebo.

Results. Baseline demographic and disease characteristics were similar across the 3 treatment groups. The median age was 48 years and 14 of 18 (78%) patients were female. The regimens were well tolerated with no reported serious adverse events; however, grade 1 or 2 injection site reactions occurred in the majority of patients receiving PVAC. The MRSS improved by 20.6% in the 15 μg PVAC arm, while it worsened by 29.8% in the placebo arm and by 16.7% in the 50 μg arm. Change in physician and patient global assessments followed similar trends.

Conclusion. In this pilot study, use of PVAC in patients with SSc appeared safe and was associated with a trend toward improved skin scores in the 15 μg treatment group. Additional evaluation of this therapeutic approach is warranted. (J Rheumatol 2005;32:2345–50)

Key Indexing Terms:
SYSTEMIC SCLEROSIS | SCLERODERMA | CLINICAL TRIAL
MODIFIED RODNAN SKIN SCORE | PVAC | THERAPY

Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by progressive thickening of the skin and variable internal organ involvement. While the course of the disease can be highly variable, it can be severely debilitating and overall survival is poor. There have been improvements in survival over the past few decades; however, this is related mainly to better management of internal organ manifestations of disease including renal crisis, pulmonary hypertension, and gastroesophageal reflux disease[1-6]. Sclerodermatous skin changes are severe, disfiguring, and lead to significant functional disability. To date, no medications have shown a clear ability to reduce or alter the progressive skin changes of SSc. There remains a large unmet clinical need for therapies addressing the underlying immunopathogenesis of SSc.

Endothelial dysfunction is suggested to be an important early pathogenic event. Studies have suggested that markers of endothelial activity or damage may be of value in monitoring disease activity and response to therapy[7]. Such markers include E-selectin, an adhesion molecule whose expression is induced on activated endothelium, and thrombomodulin, an endothelial surface glycoprotein. Elevated serum concentrations of these markers have been observed in patients with SSc, presumably as a reflection of endothelial activation or damage. It is unclear whether changes in these markers will indicate activity of fibrotic disease of the skin apart from the vasculopathy associated with SSc.

From the Division of Immunology and Rheumatology, Stanford University, Palo Alto, California; Center for Innovative Therapy, Division of Rheumatology, Allergy, and Immunology, University of California, San Diego, California; and Corixa Corporation, Seattle, Washington, USA.

Supported by Corixa Corporation, Seattle, Washington, USA.

M.C. Genovese, MD, Associate Professor of Medicine; E.F. Chakravarty, MD, Assistant Professor of Medicine; C. Thorburn, MD, Fellow, Division of Immunology and Rheumatology, Stanford University; D. Boyle, BA; Z. Tutuncu, MD; M. Halilhodzic, MD; A. Kavanaugh, MD, Professor of Medicine, Division of Rheumatology, Allergy, and Immunology, University of California; S. Kroll; J. Baughman, PhD; S. Stewart, MD, Corixa Corporation.

Address reprint requests to Dr. M.C. Genovese, 1000 Welch Road, #203, Palo Alto, CA 94304. E-mail: genovese@stanford.edu

Accepted for publication July 21, 2005.

Clinical studies in humans have evaluated heat-killed *Mycobacterium vaccae* suspensions delivered by intradermal injections as immunotherapeutic adjuncts to standard chemotherapy for tuberculosis and in other, noninfectious diseases[8-11]. Preparations of *M. vaccae* appear to be safe and well tolerated. PVAC is the denotation of a delipidated, deglycolipidated preparation of *M. vaccae*. The presumed mechanism of PVAC relates to modification of antigen-presenting cell function. Early human trials with PVAC have suggested possible efficacy in the treatment of plaque psoriasis, associated with an excellect safety profile[12,13].

Studies of PVAC in mouse models of scleroderma have shown a reduction in skin fibrosis in established cutaneous disease. The tight-skin mouse (TSK/+) model, considered particularly relevant to SSc in humans, has a point mutation in the fibrillin 1 gene that leads to dermal thickening and extensive collagen deposition in the skin, resembling the skin sclerosis detected in patients with scleroderma[14]. Ten-month-old TSK/+ mice with established disease were treated with 8 subcutaneous injections of PVAC at up to 100 μg. Each PVAC dose resulted in a statistically significant reversal of skin thickness by clinical and histological evaluation compared to saline-treated mice. A dose-response curve was seen, as 6 PVAC injections of 100 μg led to a reversal of skin thickness in most, but not all, animals, and no significant reversal of skin thickness was observed in animals receiving only 4 injections.

Based on the marked reduction in skin thickness in mouse models for SSc and the excellent safety profile with use of PVAC in both humans and animals for a variety of conditions, we investigated, in this pilot study, the safety and efficacy of 8 intradermal injections of 15 μg and 50 μg PVAC compared to placebo for clinical skin thickening in subjects with diffuse SSc.

MATERIALS AND METHODS

This pilot 24 week study utilized a randomized, double blinded, parallel group, placebo controlled design to evaluate the safety and possible biologic effect of 8 intradermal injections of PVAC in patients with diffuse SSc. The study was performed at Stanford University and the University of California San Diego with Institutional Review Board approval from each participating institution. Study drug and matching placebo were supplied by Corixa Corporation (Seattle, WA, USA).

Eligible patients were 18 years of age or older with a diagnosis of diffuse SSc and adequate renal, pulmonary, and cardiovascular function defined as serum creatinine < 2.0 mg/dl and normal urinalysis, FVC > 49% of predicted, and DLCO > 39% of predicted, and absence of uncontrolled hypertension, symptomatic coronary artery disease, pulmonary hypertension, congestive hypertension, or conduction abnormalities. Patients were required to discontinue all other treatments for scleroderma, including minocycline, cyclophosphamide, methotrexate, penicillamine, azathioprine, photopheresis, potaba, calcitriol, thalidomide, tamoxifen, oral type 1 collagen, colchicine, or prednisone > 10 mg daily, at least 28 days prior to randomization and for the duration of the 28 week study.

Exclusion criteria included diagnosis of limited SSc or other overlap connective tissue diseases, significant exposure to toxins (tainted rapeseed oil, vinyl chloride, trichlorethylene, or silica), pregnancy or lactation, unwillingness to use adequate contraception throughout the study period, current alcohol or drug abuse, current anticoagulation, or infection with human immunodeficiency virus, hepatitis B, or hepatitis C virus. Patients who required treatment with prednisone > 10 mg daily within 28 days prior to randomization were also excluded.

After informed consent was obtained, screening evaluations included complete medical history, physical examination, and screening laboratory tests including complete blood count, serum creatinine, urinalysis, and serum pregnancy test for female subjects. Pulmonary function tests, chest radiograph, and electrocardiogram (ECG) were carried out. Disease duration was measured as the time from initial physician diagnosis.

Eligible subjects were scheduled for a baseline visit within 14 days of screening. At the baseline visit, interval history and physical examination were performed. Subjects were then assessed using the Modified Rodnan Skin Score (MRSS), a validated composite measurement of skin fibrosis with a range of 0–51[15], Health Assessment Questionnaire Disability Index (HAQ-DI)[16], patient and physician global assessment by visual analog scale (VAS), measurement of hand expansion (measured in cm from tip of first to tip of fifth digit in fully extended hand), measurement of oral aperture (tip of upper incisor to tip of lower incisor), and measurements of serum E-selectin and thrombomodulin.

Patients were randomized to receive 8 doses of PVAC 15 mg, PVAC 50 mg, or placebo by intradermal injections at 3 week intervals. Administrations consisted of 0.1 ml of a solution containing PVAC 15 mg, PVAC 50 mg, or saline via superficial intradermal injection.

At each subsequent visit through Week 21, subjects were assessed for adverse events including injection site reactions, standard laboratory measures, interval history, and physical examination. Irrespective of evidence of local reaction, all prior injection sites were covered with a bandage prior to efficacy assessments. A single blinded, trained assessor evaluated all efficacy variables at each site including MRSS, HAQ-DI, VAS, hand extension, and oral aperture measurements. Injection of study drug was performed after all safety and efficacy evaluations were completed. At Week 12, subjects additionally underwent pulmonary function studies and an ECG. All safety and efficacy variables were assessed at Week 24 including pulmonary function studies and ECG. Patients were then seen at Week 28 (7 weeks after final study drug administration) for a final safety visit. Interval medical history and physical examination and repeat laboratory assessments were performed for any laboratory values that had not returned to baseline at Week 24.

Biomarkers. Serum samples were obtained at baseline and at Week 21 for determination of concentrations of E-selectin (R&D Systems, Minneapolis, MN, USA) and thrombomodulin (American Diagnostica, Stamford, CT, USA) by ELISA. Fasting samples were obtained at morning visits, and care was taken to minimize platelet activation. Serum samples were assayed in duplicate according to the manufacturer's instructions.

Statistical analysis. The study was designed to enroll 18 patients, 6 per treatment arm. This sample size was considered reasonable and feasible for a pilot study in this patient population and was not based on statistical considerations. All statistical summaries and analyses were performed on an intent-to-treat basis and included all randomized patients regardless of length of treatment. For patients who did not complete 24 weeks of the study, efficacy results from the last observation were carried forward and compared to baseline values. Baseline disease and patient characteristics were compared across treatment groups using chi-square tests for categorical data and analysis of variance for other variables. The primary efficacy endpoint, the change in MRSS from baseline to Week 24; the secondary efficacy endpoint, the best post-baseline MRSS response; and the other efficacy endpoints involving changes from baseline in oral aperture, hand extension, musculoskeletal assessment, HAQ results, patient and physician global assessments, and patient's disease-related pain assessment were performed using t tests. All comparisons of the treatment groups were performed using 2-sided tests at a 0.05 level of significance ($\alpha = 0.05$). No adjustments were made to account for multiple testing. Changes in E-selectin and thrombomodulin were assessed by ANOVA. Analyses were performed using SAS (version 8.02; SAS Institute, Cary, NC, USA).

RESULTS

Results of randomization and baseline characteristics of each group are shown in Table 1. Despite randomization, there were some notable differences between treatment arms with respect to age, disease duration, and prior therapies tried for scleroderma. This is not unexpected in a study of such small size. Patients in the placebo arm were somewhat younger than those in either treatment arm (39 vs 51 and 48 years, respectively). Mean disease duration ranged from 2.4 to 5.1 years. Patients in the PVAC 15 mg group tended to have longer disease duration and had tried more therapies for scleroderma than the other groups. The most common prior therapies across all groups included D-penicillamine (5 patients), minocycline (5 patients), methotrexate (6 patients), and prednisone (6 patients). The median baseline MRSS was 24 (range 9–38), with a trend for lower MRSS in the placebo group (median 21, range 9–26). Baseline measurements of hand extension and oral aperture were comparable among the groups.

A total of 3 patients discontinued the study prior to Week 24 (Table 2). Two patients in the PVAC 15 mg arm withdrew, one for progressive worsening of pulmonary function of > 20%, and one for perceived lack of efficacy. One subject in the PVAC 50 mg arm withdrew after 4 injections because of injection site reactions.

Safety results. In general, PVAC appeared to be safe in this population with diffuse SSc (Table 3). There were no serious adverse events during the study: no deaths, grade 4 toxicities, hospitalizations, or infections requiring intravenous antibiotics occurred. Two patients withdrew because of adverse events (one with worsening pulmonary disease, one with injection site reactions). Three patients, one from each treatment group, developed transient grade 3 toxicities (2 patients with decreases in renal function considered unrelated to study drug and one with an absolute neutrophil count < 1000), all of which resolved spontaneously.

As expected, injection site reactions appeared to be related to dose of study drug: none was reported in the placebo arm compared to occurrences in 67% of patients in the PVAC 15 mg arm and 100% of patients in the PVAC 50 mg arm. All injection site reactions were grade 1 or grade 2 and consisted mainly of localized erythema, induration, and, less commonly, mild ulceration. Most were mild and all resolved spontaneously. Other adverse events included upper respiratory infection and diarrhea.

Efficacy results. The primary efficacy endpoint of the study was the improvement in MRSS from baseline to the Week 24 assessment (Table 4). Only the PVAC 15 mg arm demonstrated improvement in MRSS, with a median decrease of 20.6%. Both the placebo and the PVAC 50 μg groups had

Table 1. Baseline characteristics.

	Placebo	PVAC 15 μg	PVAC 50 μg
Age, mean (range), yrs	39 (23–50)	51 (44–55)	48 (39–69)
Female, n (%)	5 (83)	5 (83)	4 (67)
Weight, kg, mean (range)	73 (45–104)	74 (55–98)	71 (63–80)
Disease duration, mean (range), yrs	2.4 (0.6–4.3)	5.1 (1.9–10.1)	3.0 (0.6–6.1)
No. of prior scleroderma therapies (range)	1.2 (0–3)	2.2 (0–4)	1.7 (0–3)
Modified Rodnan Skin Score (range)	19 (9–26)	26 (15–36)	27 (20–38)
Total hand extension*, cm, mean (range)	30.9 (20.0–38.0)	30.6 (20.0–43.1)	32.3 (25.0–38.2)
Oral aperture, cm, mean (range)	3.5 (2.5–5.0)	3.5 (2.0–4.0)	3.7 (2.7–4.7)
Physician global assessment, mean mm (range)	38 (6–70)	48 (24–68)	43 (21–64)
Patient global assessment, mean mm (range)	36 (12–74)	42 (22–64)	31 (2–61)
VAS Pain, mm, mean (range)	42 (9–78)	25 (7–61)	28 (16–40)
HAQ-DI	1.13	1.38	0.88

* Sum of values for left and right hands. VAS: visual analog scale, HAQ-DI: Health Assessment Questionnaire Damage Index.

Table 2. Attrition of subjects.

	Placebo	PVAC 15 μg	PVAC 50 μg
No.	6	6	6
No. discontinued prior to Week 24 (%)	0 (0)	2 (33)	1 (16)
Reason for discontinuation		(1) Worsened PFT (after Week 18)	Injection site reactions (after Week 9)
		(2) Lack of improvement (after Week 21)	

PFT: pulmonary function test.

Table 3. Adverse events.

	Placebo	PVAC 15 μg	PVAC 50 μg
Serious adverse events	0	0	0
Grade 4 toxicities	0	0	0
Grade 3 toxicities	1 (worsening renal function)	1 (ANC < 1000)	1 (worsening renal function)
Most common adverse events (%)			
Injection site conditions	0	4 (66)	6 (100)
Diarrhea	2 (33)	0	1 (16.7)
Lower extremity edema	0	2 (33)	0
Infection*	2 (33)	1 (16.7)	3 (50)
Pruritus	0	1 (16.7)	2 (33)
Epistaxis	0	2 (33)	0

* Includes upper respiratory infection, bronchitis, eye infection, nasopharyngitis and sinusitis.

Table 4. Efficacy outcomes.

Changes from Baseline	Placebo	PVAC 15 μg	PVAC 50 μg
MRSS, absolute (%)	3.5 (29.8)	–4.3 (–20.6)	3.5 (16.7)
Oral aperture, absolute, cm (%)	0	–0.1 (–3.8)	0.2 (4.8)
Hand extension, absolute, cm (%)	1.0 (3.2)	0.4 (3.8)	1.4 (4.7)
Change in HAQ-DI	0.2	–0.1	0.0
Physician global assessment, mm	–6.0	–8.5	2.5
Patient global assessment, mm	1.0	–19.0	2.0
VAS pain, mm	3.5	–4.0	–1.0

MRSS: Modified Rodnan Skin Score, HAQ-DI: Health Assessment Questionnaire Damage Index, VAS: visual analog scale.

worsening of skin disease as measured by an increase in median MRSS by 29.8% and 16.7%, respectively. The difference between the placebo and PVAC 15 μg groups was not statistically significant (p = 0.146). Secondary outcomes including median percentage change in measurement of oral aperture and hand extension showed similar results, with an improvement in or less progression of restriction in the PVAC 15 μg group compared to both the placebo group and the PVAC 50 μg group. Interestingly, patients' assessments of disease activity reflected the changes seen in measurements of skin fibrosis: perceived improvement in overall disease activity was noted by patients in the PVAC 15 μg group compared to worsening of disease activity reported by patients in the placebo or the PVAC 50 μg group. In contrast, the median physician global assessment showed, at best, modest improvements in both the placebo and PVAC 15 μg arms. There was no change in functional status as measured by the HAQ in any group. None of the comparisons with placebo was statistically significant.

E-selectin and thrombomodulin. Pre and posttreatment values for these biomarkers are shown in Table 5. For thrombomodulin, there were no significant changes pre and posttreatment in any group. For E-selectin, values after treatment decreased slightly (–5.7 ± 11.9) in the placebo group; in contrast, levels increased by 15.2 ± 23.8 in the 15 mμ group and by 14.3 ± 34.2 in the 50 μg group. None of the changes in E-selectin levels achieved statistical significance (p for trend = 0.15).

DISCUSSION

Over the past decade there have been several advances in the treatment of systemic sclerosis. Most notably there has been

Table 5. E-Selectin and thrombomodulin.

	Placebo	PVAC 15 μg	PVAC 50 μg
Thrombomodulin, ng/ml			
Pre treatment	6.6 ± 1.2	6.8 ± 1.4	6.1 ± 3.5
Post treatment	6.9 ± 1.6	7.2 ± 1.8	7.4 ± 2.7
E-selectin, ng/ml			
Pre treatment	112.5 ± 32.5	77.8 ± 18.3	82.3 ± 54
Post treatment	106.5 ± 31.2	93.01 ± 32.4	97.3 ± 33.5

the advent of therapies for the treatment of scleroderma renal crisis[2], pulmonary hypertension[3-5], and interstitial lung disease[7,17]. However, as important as these advances are in improving survival, they are targeted at preventing the worsening of end organ damage without addressing the underlying pathogenic process. These therapeutic advances have been unable to achieve significant improvement in the progressive disfiguring cutaneous process of scleroderma[1].

Current strategies to target the underlying fibrotic course of scleroderma have shown limited effect on disease progression[18-20]. Despite a lack of convincing clinical trial efficacy, immunomodulatory or immunosuppressive medications continue to be used, often because of the lack of effective alternative therapies. D-penicillamine has been studied the most extensively, with mixed results[21,22]; the most recent studies suggest that very low dose therapy was of equal utility as higher dose therapy, calling into question the true benefit of this agent[23]. Unfortunately, despite optimistic preliminary studies, recent trials of methotrexate and relaxin failed to confirm efficacy of these agents[24,25].

This study was designed to evaluate the safety and possible efficacy of PVAC for the treatment of the sclerodermatous changes of SSc. Animal models such as the TSK mouse model had suggested that this agent might be capable of reversing changes in established disease, and previous studies in human diseases such as psoriasis suggested that this approach should be safe and well tolerated. Given the lack of any data to support utility or safety for patients with more advanced internal organ disease, patients with significant internal organ disease such as renal insufficiency, pulmonary hypertension, or interstitial lung disease were excluded from participation. Further, in an attempt to create as homogeneous a population as possible, patients with limited SSc, overlap disorders, or known exposures to toxins (i.e., rapeseed oil, L-tryptophan, etc.) were also excluded. Clinical outcome measures chosen were consistent with those used in previous therapeutic trials and consistent with current consensus documents regarding validated outcomes of SSc clinical studies[1,26].

Because this was the first trial of PVAC in the treatment of human SSc, this pilot trial involved a small number of patients. Two dosing groups were chosen based on previous knowledge of the agent and ongoing clinical studies of PVAC in other disease processes (data not shown). Based on studies using PVAC in the TSK model, we estimated that a series of 8 injections would provide an adequate exposure time to the compound as well as an appropriate timeframe to assess both safety and efficacy. However, limited knowledge of effective dose, schedule, and duration of therapy needed to show improvement in human disease clearly limits the ability to draw clear conclusions about the utility of this agent in scleroderma. The overall size of the trial naturally limited the power to observe a statistically meaningful outcome even if one exists.

Despite the limitations outlined above, we did observe apparent subjective and objective improvement in patients receiving PVAC 15 μg that were not seen in patients treated in the placebo and in the PVAC 50 μg arms. The low-dose cohort had a 20% improvement in their skin score, while the placebo and high dose PVAC arm continued to show disease progression. The absence of improvement and, indeed, worsening of disease in the placebo group would be consistent with the severe and progressive nature of disease in the patient population and gives credence to the blinded design of the independent assessments. The failure to see improvement in the high-dose PVAC group does raise some concern. Our results showing a lack of a dose-response curve in this study is similar to that seen in early trials of recombinant human relaxin for the treatment of SSc, in which a reduction in skin fibrosis was seen only with the lower dose arm, while patients who received the higher dose had skin changes similar to the placebo arm[24].

Failure to see a threshold or a dose-related response could suggest that positive benefits seen in the low dose group were spurious or artifactual rather than real. It is possible that the results obtained in this study are more a reflection of the natural history of skin fibrosis in SSc than a true disease modification by PVAC therapy. The reduction of skin fibrosis in the PVAC 15 μg arm may be, at least in part, due to the longer mean disease duration of patients in this group that may be evolving into the atrophic phase of disease. In contrast, patients in the placebo and PVAC 50 μg groups have shorter mean disease duration and may be experiencing progressive fibrosis characteristic of early disease irrespective of therapeutic interventions. Additionally, changes in patient-reported outcomes, including global assessment of disease activity, could reflect regression to the mean rather than the effects of study drug. The small numbers of patients studied in this pilot trial preclude further analysis of the role of disease-specific covariates. However, despite the small number of patients and the unequal distribution of baseline characteristics despite randomization, it is also plausible that PVAC exerts pleiotropic effects at different dose levels. A larger study would be needed to determine the true efficacy of this agent at differing dose levels.

In addition, there may be a suggestion of biological activity with treatment, as indicated by a trend toward increases in the concentrations of soluble E-selectin in the active-treated groups. Both E-selectin and thrombomodulin have been shown to be overexpressed in patients with early SSc, and this has been felt to be an indication of endothelial cell activation or damage[7]. The lack of alteration in thrombomodulin argues against any direct injurious effect of treatment on the endothelium. Interestingly, E-selectin concentrations have been noted to decrease in association with clinical improvement in SSc treated with oral cyclophosphamide and alternate day prednisone[7]. With PVAC treatment we found an increase, although not statistically signif-

icant, in serum E-selectin levels, contrary to what would be expected based on studies of other agents. The significance of this finding is unclear, as patients in this study had predominately cutaneous disease and longer disease duration than the cohort in the cyclophosphamide study. We do not have data for serum E-selectin or thrombomodulin levels in healthy control subjects with which to compare baseline levels of this cohort of patients with SSc.

As a pilot study and the first use of this compound in patients with SSc, safety was of paramount concern. The side effects observed are listed in Table 3. Mild injection site reactions were reported most commonly. No severe adverse events were reported and overall PVAC appeared safe at the doses used.

Given the lack of effective therapies, and the safety profile associated with treatments currently used for scleroderma, there remains a need for safe and effective therapeutic options. While this small study had a number of limitations, objective improvement was observed in patients with SSc treated with low dose PVAC without serious or worrisome adverse events. Larger studies, adequately powered for safety and efficacy, may help to determine the effects of PVAC upon the cutaneous manifestations of SSc and to elucidate its potential as a therapeutic strategy for this disease.

REFERENCES

1. Lin ATH, Clements PJ, Furst DE. Update on disease-modifying antirheumatic drugs in the treatment of systemic sclerosis. Rheum Dis Clin North Am 2003;29:409-26.
2. Rhew EY, Barr WG. Scleroderma renal crisis: new insights and developments. Curr Rheumatol Rep 2004;6:129-36.
3. Oudiz RJ, Schilz RJ, Barst RJ, et al. Treprostinil, a prostacyclin analogue, in pulmonary arterial hypertension associated with connective tissue disease. Chest 2004;126:420-7.
4. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet 2001;358:1119-23.
5. Badesch DB, Tapson VF, McGoon MD, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. Ann Intern Med 2000;132:425-34.
6. Young MA, Rose S, Reynolds JC. Gastrointestinal manifestations of scleroderma. Rheum Dis Clin North Am 1996;22:797-823.
7. Apras S, Ertenli I, Ozbalkan Z, et al. Effects of oral cyclophosphamide and prednisolone therapy on the endothelial functions and clinical findings in patients with early diffuse systemic sclerosis. Arthritis Rheum 2003;48:2256-61.
8. Onybujoh PC, Abdulmumini T, Robinson S, Rook GAW, Stanford JL. Immunotherapy with Mycobacterium vaccae as an addition to chemotherapy for the treatment of pulmonary tuberculosis under difficult conditions in Africa. Respir Med 1995;89:199-207.
9. Stanford JL, Stanford CA. Immunotherapy of tuberculosis with Mycobacterium vaccae NCTC 11659. Immunobiology 1994;191:555-63.
10. Lehrer A, Bressanelli A, Wachsmann V, et al. Immunotherapy with Mycobacterium vaccae in the treatment of psoriasis. FEMS Immunol Med Microbiol 1998;21:71-7.
11. Rambukkana A, Das PK, Witkamp L, Yong S, Meinardi MMHM, Bos JD. Antibodies to mycobacterial 65-kDa heat shock protein and other immunodominant antigens in patients with psoriasis. J Invest Dermatol 1993;100:87-92.
12. Balagon MV, Tan PL, Prestidge R, et al. Improvement in psoriasis after intradermal administration of delipidated, deglycolipidated Mycobacterium vaccae (PVAC): results of an open-label trial. Clin Exp Dermatol 2001;26:233-41.
13. Dalbeth N, Yeoman S, Dockerty JL, et al. A randomised placebo controlled trial of delipidated, deglycolipidated Mycobacterium vaccae as immunotherapy for psoriatic arthritis. Ann Rheum Dis 2004;63:718-22.
14. Pablos JL, Everett ET, Norris JS. The tight skin mouse: an animal model of systemic sclerosis. Clin Exp Rheumatol. 2004;22 Suppl 33:S81-5.
15. Furst DE, Clements PJ, Steen VD, et al. The modified Rodnan skin score is an accurate reflection of skin biopsy thickness in systemic sclerosis. J Rheumatol 1998;25:84-8.
16. Clements PJ, Wong WK, Hurwitz EL, et al. Correlates of the disability index of the Health Assessment Questionnaire: a measure of functional impairment in systemic sclerosis. Arthritis Rheum 1999;42:2372-80.
17. White B, Moore WC, Wigley FM, Xiao HQ, Wise RA. Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. Ann Intern Med 2000;132:947-54.
18. Furst DE, Clements PJ, Hillis S, et al. Immunosuppression with chlorambucil, versus placebo, for scleroderma. Results of a three-year, parallel, randomized, double-blind study. Arthritis Rheum 1989;32:584-93.
19. Jansen GT, Barraza DF, Ballard JL, Honeycutt WM, Dillaha CJ. Generalized scleroderma. Treatment with an immunosuppressive agent. Arch Dermatol 1968;97:690-8.
20. Casas JA, Saway PA, Villarreal I, et al. 5-fluorouracil in the treatment of scleroderma: a randomised, double blind, placebo controlled international collaborative study. Ann Rheum Dis 1990;49:926-8.
21. Steen VD, Medsger TA Jr, Rodnan GP. D-penicillamine therapy in progressive systemic sclerosis (scleroderma): a retrospective analysis. Ann Intern Med 1982;97:652-9.
22. Jimenez SA, Sigal SH. A 15-year prospective study of treatment of rapidly progressive systemic sclerosis with D-penicillamine. J Rheumatol 1991;18:1496-503.
23. Clements PJ, Furst DE, Wong WK, et al. High-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis: analysis of a two-year, double-blind, randomized, controlled clinical trial. Arthritis Rheum 1999;42:1194-203.
24. Seibold JR, Korn JH, Simms R, et al. Recombinant human relaxin in the treatment of scleroderma. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2000;132:871-9.
25. Pope JE, Bellamy N, Seibold JR, et al. A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. Arthritis Rheum 2001;44:1351-8.
26. Merkel PA, Clements PJ, Reveille JD, Suarez-Almazon ME, Valentini G, Furst D. Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6. J Rheumatol 2003;30:1630-47.