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LOSARTAN THERAPY FOR RAYNAUD'S PHENOMENON AND SCLERODERMA

Clinical and Biochemical Findings in a Fifteen-Week, Randomized, Parallel-Group, Controlled Trial

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Objective. **To compare the efficacy and tolerability of losartan, an antagonist of angiotensin II receptor type 1, with nifedipine for the treatment of primary and secondary Raynaud's phenomenon (RP) in a pilot study.**

Methods. **In a randomized, parallel-group, controlled trial, patients with primary RP (n = 25) or RP secondary to systemic sclerosis (SSc [scleroderma]; n = 27) were allocated to receive 12 weeks' treatment with either losartan (50 mg/day) or nifedipine (40 mg/day). Primary outcome variables were the severity and frequency of RP episodes and findings on vascular measurements, including thermography and laser Doppler flowmetry. Serum levels of soluble adhesion molecules, endothelin 1, fibrinogen, von Willebrand factor, and procollagen type I N-terminal propeptide (PINP) were also measured.**

Results. **There was a reduction in the severity of RP episodes following treatment with losartan and with nifedipine, but this effect was greater in the losartan arm of the study ($P < 0.05$): episode frequency was reduced only in the losartan group ($P < 0.01$ versus baseline). Symptomatic improvement was associated with a significant reduction in soluble vascular cell adhesion molecule 1 and PINP ($P < 0.01$). Subgroup analysis suggested that although these biochemical changes occurred mainly in SSc patients, the clinical benefit was greater in the primary RP group.**

Conclusion. **This study confirms the tolerability of short-term treatment of RP with losartan, and our data suggest its clinical benefit. Further evaluation of this drug as a long-term treatment for SSc-associated RP should be considered, since it may have additional disease-modifying potential.**

Episodic digital ischemia provoked by cold and emotion was first described by Maurice Raynaud over 130 years ago (1). When it occurs in isolation, it is designated primary Raynaud's phenomenon (RP) to distinguish it from those cases in which there is an underlying or associated pathology. It affects ~10% of the adult population, with a predilection for females, and up to 5% of patients presenting with this condition later develop an autoimmune rheumatic disorder, such as systemic sclerosis (SSc; scleroderma) (2). Successful treatment is often difficult, and although clinical trials suggest that vasodilators can be effective, the responses of individual patients to a particular agent are often idiosyncratic.

The pathogenesis of the vascular dysfunction underlying RP is incompletely understood, and relatively few well-controlled clinical trials with vasoactive drugs have been undertaken. Lack of distinction between primary and secondary RP in some of these studies makes their interpretation difficult. Calcium channel antagonists such as nifedipine have been shown to be effective (3–6), although striking differences in individual responses have been described (3,7). Moreover, efficacy is often particularly limited in patients with RP

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secondary to SSc (8), and side effects such as dizziness, hypotension, ankle edema, and headache are common (9,10). There is therefore a need to evaluate alternative treatments that might be more effective or have a better side-effect profile.

Losartan is a specific nonpeptide angiotensin II type 1 receptor antagonist (11) that has been successfully used to treat systemic hypertension (12). It does not exhibit the adverse respiratory side effects, such as cough, that are common to angiotensin-converting enzyme (ACE) inhibitors (13), which have also been shown to be of controversial benefit in RP (14). A small number of patients with primary RP have been treated with low doses of losartan, and a significant improvement in RP episodes was demonstrated in that study (15). The efficacy of losartan in secondary RP has not been studied, although 1 case report did not demonstrate its benefit in the treatment of established scleroderma renal crisis (16).

Here we report the results of a pilot study comparing the tolerability and efficacy of losartan with nifedipine in a group of patients with primary RP or RP secondary to SSc. The effect of these drugs on serum markers of vascular damage and connective tissue turnover was also investigated to evaluate their possible disease-modifying potential.

Table 1. Baseline characteristics of losartan and nifedipine treatment groups*

	Losartan	Nifedipine	Total
Demographic variables			
No. of patients enrolled	26	26	52
No. of females:males	22:4	17:9	39:13
PRP	10:2	5:8	15:10
SSc	12:2	12:1	24:3
Median age, years (range)	51 (21–62)	51 (19–68)	51 (19–68)
PRP	49 (21–61)	49 (19–63)	49 (19–63)
SSc	55 (30–62)	53 (36–68)	54 (30–68)
No. of current smokers	3	3	6
No. of ex-smokers	4	5	9
Clinical characteristics			
No. with PRP	12	13	25
No. with SSc	14	13	27
lcSSc	5	13	18
dcSSc	9	0	9
No. of patients who have already tried nifedipine	6	4	10
No. of patients who have tried other vasodilators	10	10	20
No. of patients who have not tried any vasodilators	10	12	22

* PRP = primary Raynaud's phenomenon; SSc = systemic sclerosis; lcSSc = limited cutaneous systemic sclerosis; dcSSc = diffuse cutaneous systemic sclerosis.

PATIENTS AND METHODS

Study design. This was a randomized, parallel-group study of 15 weeks' duration. The study was performed over 1 winter to minimize seasonal effects on the severity and frequency of RP episodes. Patients stopped taking vasodilator drugs 3 weeks prior to entry into the trial (week 0) and underwent baseline medical assessment, including medical history and physical examination.

Graded nailfold capillaroscopy was used at baseline as a confirmatory test for primary RP; the abnormalities were quantified using a standard scale as grade I, II, or III (17). Vascular evaluation, including cold-challenge infrared thermography and laser Doppler flowmetry (LDF), were performed at baseline (week 3) and at the end of treatment (week 15). Serum and plasma samples were taken before and at the completion of therapy.

Patients were randomized to receive either nifedipine or losartan for 12 weeks. Nifedipine was formulated as 20-mg nifedipine retard tablets and were taken twice a day. Losartan was formulated as 50-mg tablets (Merck, Sharp and Dohme, Hertford, UK) and were taken once a day. The relatively low dosage of nifedipine was to minimize side effect–related withdrawals from the control group.

Patients. Sixty patients from the outpatient clinic at the Rheumatology Department, Royal Free Hospital, who had confirmed RP were screened for the study. Fifty-two patients were recruited into the study; 8 patients did not fulfil the inclusion criteria. The demographic characteristics of the study patients are summarized in Table 1.

RP was diagnosed on the basis of a history of episodic digital vasospasm with triphasic color changes. For inclusion in the study, patients had to have, on average, at least 6 episodes of RP per week. Absence of rheumatoid factor, antinuclear antibodies, or disease-specific antibodies (anticentromere, anti–Scl-70, anti–U3 RNP, and anti–polymerase I and III antibodies), as well as absence of significant nailfold capillaroscopic abnormalities were required for inclusion to the primary RP group.

Patients with features of undifferentiated connective tissue disease were not recruited. Patients under the age of 18 or over the age of 70 and women of childbearing age who were not using adequate contraception were excluded. Patients with a history of significant cardiorespiratory disease or renal impairment and those who were taking ACE inhibitors were also ineligible. Five of the patients who were screened had either positive autoantibodies (4 patients) or a capillary score of III (1 patient) in the absence of a defined connective tissue disease. Since these cases, which are sometimes designated "autoimmune RP," may represent a distinct clinical group, these 5 patients were not included in the trial.

Of the patients entered into the study, 25 (10 males and 15 females; median age 49 years, range 19–63) had primary RP. The remaining 27 patients (3 males and 24 females; median age 54 years, range 30–68) had RP secondary to SSc, as classified by the preliminary criteria of the American College of Rheumatology (formerly, the American Rheuma-

tism Association) (18). Eighteen patients had limited cutaneous SSc (lcSSc) and 9 had the diffuse cutaneous SSc (dcSSc).

Patients with primary and secondary RP were separately randomized to receive 1 of the 2 drugs to maintain equal proportions in the 2 treatment arms. Ethical approval for the study was obtained from the Royal Free Hospital Ethical Practices Committee, and informed consent was given by all patients.

Assessment of RP symptoms. Patients were given symptom diaries and asked to select 1 day each week for recording their symptoms throughout the 12 weeks of treatment. An episode of RP was defined as the occurrence of pallor followed by cyanosis, with or without associated pain. Patients recorded symptom severity using a visual analog scale of 0–10, where 10 = the worst episode ever experienced and 0 = no episodes. The daily frequency of RP episodes was documented on the same day. Symptom diaries were collected at the last visit. Patients were asked to report any adverse events occurring during the study.

Noninvasive vascular studies. At baseline and after treatment, the response of each patient to a mild cold challenge of the hands in water was assessed using the techniques of LDF and infrared thermography. All participants were asked to avoid alcohol for 24 hours prior to the measurements, as well as hot caffeinated drinks and hot meals on the day of the vascular tests. LDF refers to the measurement of the flux of moving red blood cells in a volume of skin beneath a measurement probe, and is derived from the Doppler shift of the frequency of laser light as it scatters from moving erythrocytes. LDF flux is measured in arbitrary units (AU). Infrared thermography estimates skin temperature using a thermal imaging camera.

At each visit, the subject sat comfortably in a temperature-controlled room (within 1°C of 23°C) for 15 minutes before measurements were taken. Laser Doppler skin probes were attached to the pulp of each middle finger. An initial thermal image of both hands was recorded using the Starsight Thermal Camera (Insight Vision Systems, Malvern, UK). Laser Doppler flux was then recorded continuously for a period of 5 minutes to ensure that skin flux had reached a stable level during the equilibration period prior to measurement (MBF3D Dual Channel Blood Flow Monitor; Moor Instruments, Devon, UK). This recording period was extended, where necessary, until stable flux readings from both fingers were observed for a period of 2 minutes.

Prior to mild cold challenge of the hands, a further thermal image was recorded. Both hands were then gloved, leaving the laser Doppler probes in place, and immersed in water at 15°C for 1 minute. After cold challenge, the hands were returned to their original positions, and thermal images were taken at 1-minute intervals for a 10-minute period. The laser Doppler trace was recorded from both fingers throughout this period.

Laser Doppler settings for all subjects were as follows: time constant 1 second and bandwidth 14.9 kHz. Laser Doppler traces were transferred to a personal computer for further analysis. The LDF mild cold challenge recovery curves from each hand were analyzed by calculating the average values of flux over a 10-second period at 3 time points: just prior to cold challenge and immediately after, 5 minutes after, and 10 minutes after cold challenge. The thermographic images were analyzed (Thermosoft image analysis software package; Moor Instruments) by computing the mean fingertip temperature (excluding the thumbs) at the same time points as for LDF.

Serum and plasma markers. Blood samples were collected immediately before and on completion of therapy. Serum and plasma were obtained by centrifugation of whole blood at 3,000*g* for 10 minutes, and aliquots were stored at −20°C. To investigate changes in underlying disease mechanisms and to identify serologic factors for assay in future studies, biochemical markers previously suggested to be abnormal in patients with SSc or RP were measured. Thus, levels of circulating soluble markers of endothelial cell function, including soluble isoforms of intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), E-selectin (E-selectin), endothelin 1 (ET-1), von Willebrand factor (vWF), and fibrinogen, as well as procollagen type I N-terminal propeptide (PINP) were evaluated.

Table 2. Effect of losartan or nifedipine therapy on clinical variables*

	Losartan				Nifedipine			
End point, group	Week 3, mean ± SD	Week 15, mean ± SD	*P,* week 3 vs. week 15	% of baseline†	Week 3, mean ± SD	Week 15, mean ± SD	*P,* week 3 vs. week 15	% of baseline†
Severity of RP episode (0–10 scale)								
Whole group	5.50 ± 2.46	2.84 ± 2.40	0.0003‡	51§	4.48 ± 2.40	3.90 ± 2.77	0.49	82
SSc patients	5.54 ± 2.79	3.77 ± 2.40	0.064	64§	4.62 ± 2.40	4.12 ± 2.55	0.63	91
PRP patients	5.46 ± 2.22	1.83 ± 2.04	0.002‡	38§	4.25 ± 2.54	3.56 ± 3.22	0.64	70§
P (SSc vs. PRP)	0.94				0.75			
Frequency of RP episode (no. of episodes/day)								
Whole group	3.52 ± 2.16	1.96 ± 1.90	0.009‡	50§	3.65 ± 3.01	4.40 ± 4.17	0.52	154
SSc patients	4.31 ± 2.63	2.62 ± 2.56	0.091	55§	3.92 ± 2.57	4.17 ± 2.73	0.82	156
PRP patients	2.67 ± 1.07	1.25 ± 1.14	0.005‡	45§	3.25 ± 3.73	4.75 ± 5.95	0.56	151
P (SSc vs. PRP)	0.055				0.67			

* RP = Raynaud's phenomenon; SSc = systemic sclerosis; PRP = primary Raynaud's phenomenon.
† Mean of individual patients, each expressed as a percentage of his or her own baseline.
‡ $P \leq 0.01$, by paired *t*-test.
§ ≥20% reduction.

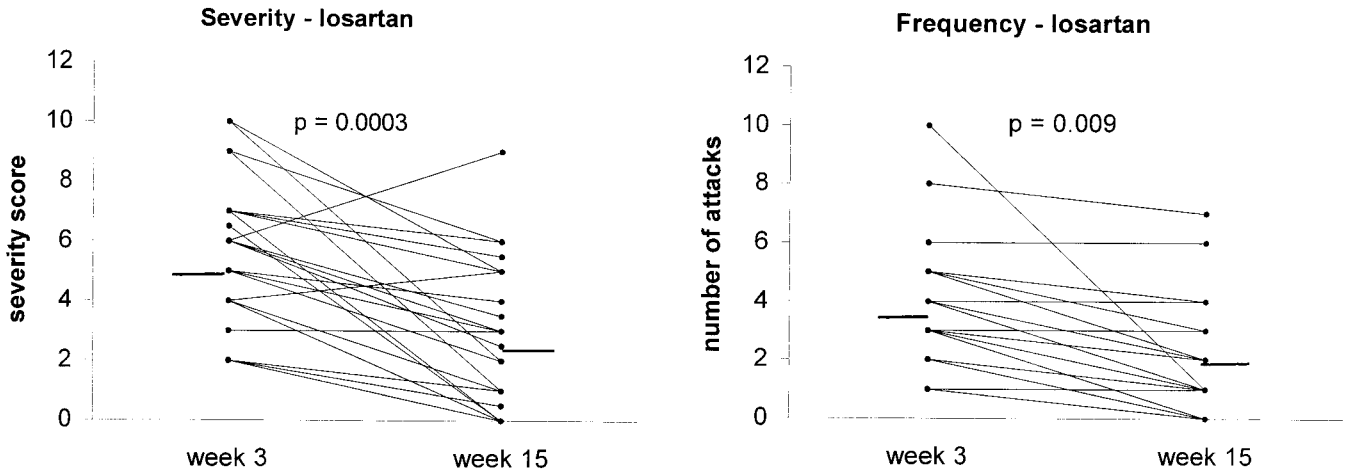

Figure 1. Effect of losartan on the raw severity score and frequency of episodes of Raynaud's phenomenon. The data points for each patient are connected by a line. Some patients had identical responses; therefore, the number of lines is fewer than the number of patients in each group. Short horizontal lines show the group mean. *P* values determined by paired *t*-test.

Commercially available enzyme-linked immunosorbent assay (ELISA) test kits were used for sICAM-1, sVCAM-1, and E-selectin (R&D Systems, Oxford, UK) and for ET-1 (Cozart Bioscience, Abingdon, UK). Serum concentrations of PINP were evaluated using a commercially available radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). Fibrinogen was measured by a modified Clauss technique using bovine thrombin and a Thromboscreen T400C coagulometer (both from Pacific Hemostasis, Huntersville, NC). Levels of vWF were determined by ELISA according to a previously described assay (19).

Statistical analysis. Clinically significant improvement in RP episodes was defined as a 20% improvement in the variables documented in the symptom diaries at baseline. Severity and frequency scores for baseline (week 3) and for each of weeks 6, 9, 12, and 15 during treatment were assessed by analysis of variance (ANOVA). Similarly, severity and frequency scores normalized to 100% at baseline were also subject to ANOVA. Differences in the frequencies of adverse effects were tested by chi-square test. Pre- and posttreatment values of the biochemical variables were compared using Student's paired *t*-test to assess the impact of therapy. The laser Doppler and thermographic data were analyzed by the use of ANOVA, followed, where appropriate, by Student's *t*-test to compare groups. Where multiple testing was carried out, a more rigorous statistical significance was used ($P < 0.01$, rather than $P < 0.05$).

RESULTS

Clinical variables and serum markers. Baseline demographic and clinical variables for the 2 treatment arms are shown in Table 1. There were no statistically significant differences between the 2 treatment groups, save in the distribution of lcSSc and dcSSc ($P < 0.006$, by chi-square test). In particular, the preponderance of men in the nifedipine group was not statistically significant.

Comparison of the variables at baseline with those after the 12-week treatment period is shown in Table 2. These data demonstrate the mean change from baseline together with the mean percentage of change over the study period. Analysis of the symptom diaries revealed a significant mean reduction in RP severity (49%; $P = 0.0003$) and frequency of episodes (50%; $P = 0.009$) over the treatment period for patients receiving losartan (Figure 1). Although in the nifedipine group, there was a mean reduction of 18% in RP severity (Figure 2), this was not statistically significant ($P = 0.49$). There was also a nonsignificant increase in the frequency of RP episodes in this group ($P = 0.52$).

An idiosyncratic response of patients to nifedipine treatment was seen in this study. Overall, for nifedipine, 11 of 26 patients showed improvement in the severity score between weeks 3 and 15, compared with 21 of 26 for losartan ($P < 0.04$, by chi-square test). Similarly, for frequency of episodes, only 7 of 20 patients improved with nifedipine therapy, whereas 18 of 25 improved with losartan ($P < 0.02$, by chi-square test). When primary RP and SSc groups were considered separately, the only significant finding was for the frequency of RP episodes in the primary RP group: 9 of 12 patients taking losartan, but only 2 of 13 patients taking nifedipine, showed improvement ($P < 0.03$, by chi-square test).

Figures 3 and 4 are plots of normalized severity and frequency scores. These data were subjected to ANOVA, and significant effects ($P \leq 0.05$) were investigated using the unpaired *t*-test. The improvement in the severity of the episodes in the losartan group was

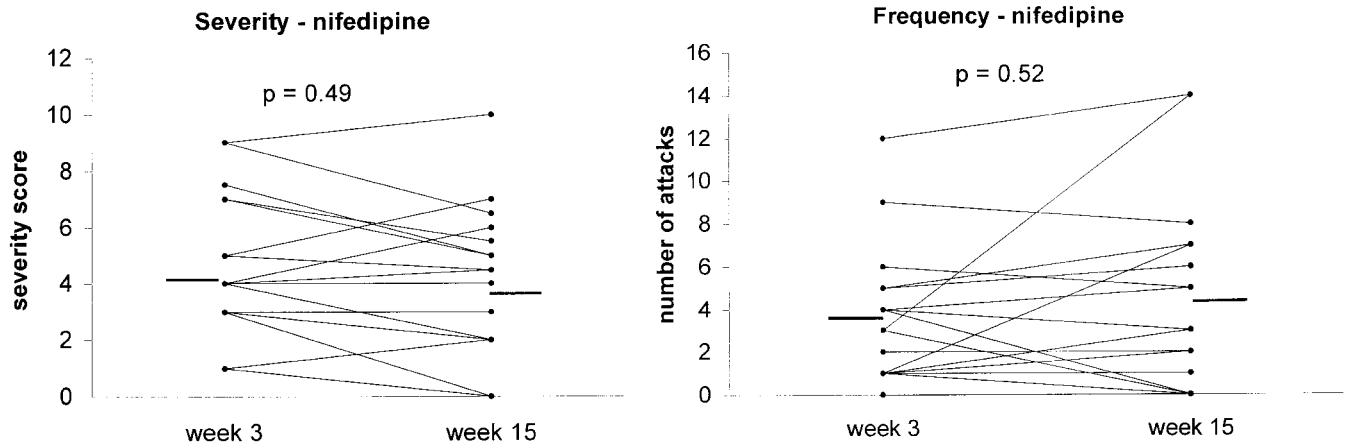

Figure 2. Effect of nifedipine on the raw severity score and frequency of episodes of Raynaud's phenomenon. The data points for each patient are connected by a line. Some patients had identical responses; therefore, the number of lines is fewer than the number of patients in each group. Short horizontal lines show the group mean. *P* values determined by paired *t*-test.

observed after 3 weeks of treatment (week 6 of the study) and subsequently enhanced, with a significant reduction ($P < 0.002$, by *t*-test) in the score at week 15 (Figure 3). A reduction in the severity score in the nifedipine group was observed only after 9 weeks of treatment (week 12 of the study) (Figure 3), but did not reach significance by week 15. The difference between groups was significant at week 15 ($P < 0.05$, by *t*-test).

The improvement in the episode frequency, as mentioned above, was observed only in the losartan group. This occurred between weeks 9 and 12 and was maintained at the end of treatment (Figure 4). There was a statistically significant difference between treatments at week 15 ($P < 0.02$, by *t*-test). However, no steady state in the normalized severity and frequency scores at week 15 was reached in any of the groups (Figures 3 and 4).

This improvement in symptoms in patients receiving losartan was accompanied by a significant reduction in serum levels of circulating PINP ($P = 0.009$) and VCAM-1 ($P = 0.003$) (Figure 5 and Table 3). Similar trends were observed for ET-1 and sICAM-1 (18% and 11% reduction in posttreatment levels, respectively). Nifedipine significantly reduced serum concentrations of sVCAM-1 only ($P = 0.01$), with no effect on other biochemical variables. There was no reduction in the

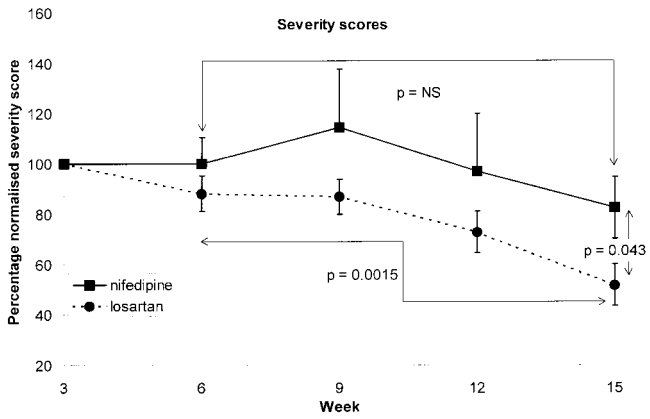

Figure 3. Effect of losartan and nifedipine on the severity of episodes of Raynaud's phenomenon. The severity score was normalized to 100% at baseline. Bars show the mean and 1 SEM. *P* values determined by unpaired *t*-test. NS = not significant.

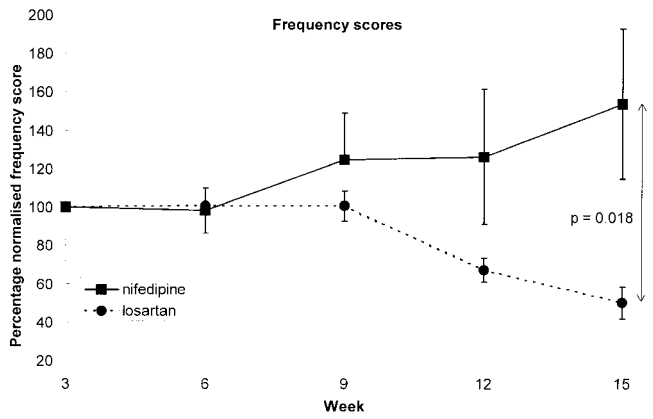

Figure 4. Effect of losartan and nifedipine on the frequency of episodes of Raynaud's phenomenon. The severity score was normalized to 100% at baseline. Bars show the mean and 1 SEM. *P* values determined by unpaired *t*-test.

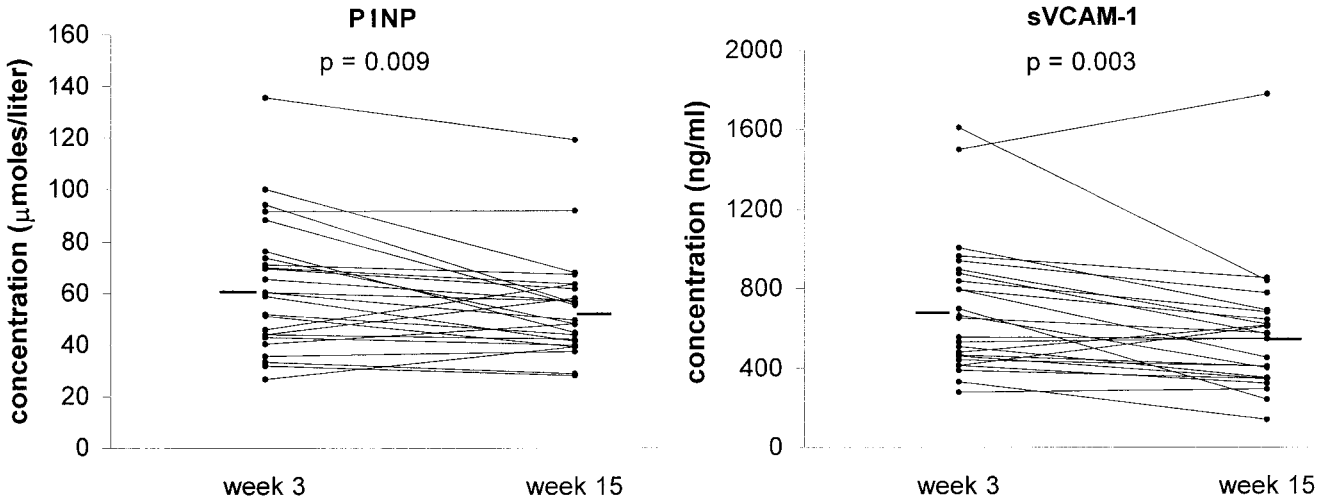

Figure 5. Serum levels of procollagen type I N-terminal propeptide (PINP) and soluble vascular cell adhesion molecule 1 (sVCAM-1) before and after treatment with losartan. The data points for each patient are connected by a line. Some patients had identical responses; therefore, the number of lines is fewer than the number of patients in each group. Short horizontal lines show the group mean. *P* values determined by paired *t*-test.

circulating levels of soluble E-selectin, vWF, and fibrinogen in either treatment arm. The magnitude of the effects of losartan and nifedipine in down-regulating biochemical variables was analyzed by unpaired *t*-test and showed significantly greater changes in sICAM-1 ($P = 0.01$) and PINP ($P = 0.01$) with losartan therapy.

The results from noninvasive vascular studies did not reflect changes in clinical and biochemical variables.

Table 3. Effect of losartan or nifedipine therapy on biochemical variables*

Variable, treatment	Week 3 (before tx), mean ± SD	Week 15 (after tx), mean ± SD	Week 3 to week 15, mean	Week 15 mean as % of week 3 mean	*P*, by paired *t*-test (within drug)	*P*, by unpaired *t*-test (between drugs)
sICAM-1 (ng/ml)						
Losartan	321.9 ± 141	285.4 ± 160	36.5	89	0.04	
Nifedipine	294.8 ± 276	313.5 ± 310	−18.6	106	0.13	0.01†
sVCAM-1 (ng/ml)						
Losartan	701 ± 334.5	561 ± 314	139	80	0.003†	
Nifedipine	822.3 ± 463	590.6 ± 354	231.6	72‡	0.01†	0.32
sE-selectin (ng/ml)						
Losartan	39.06 ± 17	37.5 ± 16	1.55	96	0.32	
Nifedipine	49.17 ± 29	49.25 ± 26	−0.79	100	0.97	0.59
Endothelin 1 (fmoles/ml)						
Losartan	0.516 ± 0.44	0.421 ± 0.37	0.097	82	0.07	
Nifedipine	0.592 ± 0.44	0.573 ± 0.54	0.018	97	0.83	0.65
vWF (IU/dl)						
Losartan	49.8 ± 29.04	52.9 ± 31.54	−3.12	106	0.65	
Nifedipine	56.15 ± 33.7	50.1 ± 24.88	6.05	89	0.19	0.26
Fibrinogen (μmoles/liter)						
Losartan	1.44 ± 0.43	1.47 ± 0.52	−0.03	102	0.69	
Nifedipine	1.35 ± 0.4	1.32 ± 0.43	0.03	98	0.76	0.62
PINP (μg/liter)						
Losartan	62.45 ± 52.5	53.9 ± 19.5	8.48	86	0.009†	
Nifedipine	54.2 ± 25.9	54.9 ± 26.9	−0.65	101	0.73	0.01†

* tx = treatment; sICAM-1 = soluble intercellular adhesion molecule 1; sVCAM-1 = soluble vascular cell adhesion molecule 1; sE-selectin = soluble E-selectin; vWF = von Willebrand factor; PINP = procollagen type I N-terminal propeptide.
† $P \leq 0.01$, by paired *t*-test.
‡ ≥20% reduction from baseline.

Table 4. Comparison of baseline data for patients with primary Raynaud's phenomenon and patients with systemic sclerosis*

Variable	PRP		SSc		*P,* by unpaired *t*-test (between diseases)
	Mean ± SD	Range	Mean ± SD	Range	
sICAM-1 (ng/ml)	268.6 ± 111	147–589	352.6 ± 263	134–1,364	0.06
sVCAM-1 (ng/ml)	600.6 ± 463	251–1,428	891.4 ± 334	440–2,050	0.007†
sE-selectin (ng/ml)	37.7 ± 16.5	14.9–80.2	48.3 ± 27	13.15–105	0.06
Endothelin 1 (fmoles/ml)	0.48 ± 0.41	0.14–1.48	0.61 ± 0.46	0.17–1.49	0.18
vWF (IU/dl)	50.2 ± 36.5	23–162	54.56 ± 26.5	23–177	0.65
Fibrinogen (μmoles/liter)	1.48 ± 0.32	0.99–2.63	1.33 ± 0.42	1–2.18	0.21
PINP (μg/liter)	49.5 ± 19.9	26.7–94	66.22 ± 27.8	27–135.6	0.02

* See Table 3 for definitions of abbreviations.
† $P \leq 0.01$, by unpaired *t*-test.

Thus, there was no improvement in recovery 10 minutes after cold challenge, as assessed by thermography, nor any increase in LDF flux in any treatment group.

Adverse events. Adverse events were more common among the patients taking nifedipine compared with those taking losartan. Side effects of treatment were reported by 10 of 26 patients (39%) receiving nifedipine and 3 of 26 patients (12%) receiving losartan. This difference was statistically significant ($P < 0.005$).

Well-known side effects such as headache, flushing, nausea, and ankle swelling were reported by patients in the nifedipine group and led to the withdrawal of 4 patients (15%): 3 patients because of severe headaches, which did not dissipate in the first 4 days of treatment, and 1 patient because of persistent ankle swelling. These patients were subsequently treated with alternative vasodilators.

Occasional dizziness was reported by 3 of 26 patients (12%) in the losartan group. One SSc patient experienced pleuritic-type chest pain during the second week of treatment with losartan and was withdrawn from the study. Clinical examination, blood tests, electrocardiogram, and chest radiographs did not show any abnormality other than those related to SSc. Therefore, this adverse event was unlikely to have been caused by the study treatment.

Forty-five of the 52 patients (87%) completed the trial. The number of patients withdrawing from the study was significantly higher in the nifedipine group (6 of 26 patients; 23%) compared with the losartan group (1 of 26 patients; 4%) ($P < 0.02$, by chi-square test). Side effects in 4 patients taking nifedipine (15%) and 1 patient taking losartan (4%) led to premature discontinuation and study withdrawals. Two patients from the nifedipine group (8%) failed to complete the trial because of inefficacy of the treatment. All patients who withdrew discontinued treatment during the first 2 weeks of study without providing completed diaries.

Comparative responses for primary and secondary RP. Baseline variables were compared between patients with primary RP and SSc. These data are summarized in Tables 2 and 4. At baseline, SSc patients reported a greater frequency of RP episodes, which just failed to reach significance ($P < 0.06$). There was no significant difference in episode severity between the disease groups. Analysis of baseline biochemical variables revealed significantly higher concentrations of sVCAM-1 ($P = 0.007$) and PINP ($P = 0.02$) and the same trend for sICAM-1, E-selectin, and to a lesser extent, ET-1. No significant differences were observed for fibrinogen and vWF.

The baseline mean hand temperature was lower in the SSc group (mean ± SEM 28.4 ± 0.6°C) than in the primary RP group (29.5 ± 0.8°C), but this difference was not significant. At baseline (week 3), laser Doppler flux before cold challenge was significantly lower in the SSc group (mean ± SD 137 ± 92 AU) than in the primary RP group (269 ± 187 AU) ($P = 0.009$, by unpaired *t*-test).

Subgroup analysis revealed some differences in the clinical and biochemical treatment effects (Tables 2 and 5). Generally, a better symptomatic response was observed at week 15 in patients with primary RP in both treatment arms. In the losartan-treated group, the difference was highly significant, with a decrease of 62% in the mean episode severity ($P = 0.002$) and decrease of 55% in frequency ($P = 0.005$). In the nifedipine group, the 30% decrease in mean episode severity in patients with primary RP was not significant ($P = 0.06$). There was a nonsignificant increase in episode frequency in this group (Table 2). In SSc patients at week 15, there were neither clinically nor statistically significant changes in

Table 5. Effect of treatment on end point evaluation for patients with systemic sclerosis and patients with primary Raynaud's phenomenon*

End point, group	Losartan				Nifedipine			
	Week 3, mean ± SD	Week 15, mean ± SD	% of baseline†	*P*	Week 3, mean ± SD	Week 15, mean ± SD	% of baseline†	*P*
sICAM-1 (ng/ml)								
SSc	376 ± 159	362 ± 178	96	0.68	331 ± 335	356 ± 396	107	0.21
PRP	291 ± 132	236 ± 125	81	0.0005‡	258 ± 71	242 ± 79	93	0.72
sVCAM-1 (ng/ml)								
SSc	837 ± 366	650 ± 384	78§	0.03	950 ± 555	809 ± 399	85	0.03
PRP	554 ± 228	465 ± 186	84	0.05	671 ± 357	455 ± 271	67	0.03
sE-selectin (ng/ml)								
SSc	44.0 ± 20.0	43.4 ± 17.8	100	0.8	52.8 ± 33	53.5 ± 27	101	0.87
PRP	38.2 ± 19.5	43.9 ± 31.8	83	0.5	43.7 ± 22	42.9 ± 23	98	0.5
Endothelin 1 (fmoles/ml)								
SSc	0.60 ± 0.50	0.51 ± 0.47	85	0.2	0.6 ± 0.42	0.56 ± 0.40	93	0.29
PRP	0.42 ± 0.36	0.32 ± 0.17	76§	0.24	0.57 ± 0.48	0.58 ± 0.73	101	0.96
vWF (IU/dl)								
SSc	50.1 ± 6.7	58.8 ± 22.2	117	0.17	49.4 ± 42.3	46.6 ± 39.3	94	0.82
PRP	59.3 ± 37.8	55.1 ± 27.7	93	0.54	51.4 ± 28.2	42.6 ± 19.2	83	0.15
Fibrinogen (μmoles/liter)								
SSc	1.35 ± 0.46	1.56 ± 0.58	115	0.13	1.31 ± 0.49	1.35 ± 0.39	103	0.74
PRP	1.52 ± 0.38	1.37 ± 0.45	90	0.25	1.42 ± 0.22	1.27 ± 0.50	89	0.39
PINP (μg/liter)								
SSc	68.9 ± 27	58.5 ± 20.7	85	0.03	63.3 ± 29.4	64.9 ± 30.2	102	0.55
PRP	55.4 ± 22.9	49.0 ± 17.6	88	0.17	40.6 ± 10.4	39.8 ± 10.2	98	0.76

* See Table 3 for definitions of abbreviations.
† Mean of individual patients, each expressed as a percentage of his or her own baseline.
‡ $P \leq 0.01$, by paired *t*-test.
§ ≥20% reduction.

episode frequency or severity with nifedipine. In contrast, losartan produced clinically significant reductions in both frequency and severity (45% and 36% decrease, respectively), although these just failed to reach statistical significance (Table 2).

The only statistically significant changes in serum markers in the primary RP and SSc groups, when considered separately (Table 5), was a decrease in sICAM-1 in the primary RP group treated with losartan ($P = 0.0005$). However, there was a reduction in sVCAM-1 in all subsets, as well as a reduction in PINP in SSc patients treated with losartan (Table 5). No appreciable differences were observed in soluble E-selectin, fibrinogen, vWF, and ET-1 levels.

DISCUSSION

The overall improvement in the severity and frequency of episodes of RP in losartan-treated patients is encouraging. Although subjective, self-reporting of RP symptoms appears to be a reliable assessment tool, particularly when combined with longitudinal analysis of the data (20). The results for serum markers provide additional indirect evidence that losartan could benefit the underlying pathologic processes in individuals with secondary RP. A steady state did not appear to have been reached at the end of this study, and it is possible that a greater benefit might be achieved after more prolonged administration. Although most studies evaluating the effect of drugs on RP symptoms are also performed over 6–12-week periods, longer-term trials of losartan may be worthwhile.

An unblinded design is a significant limitation of our study, increasing the likelihood of a placebo response to a perceived novel treatment. However, reported placebo responses for clinical outcome variables in other RP studies is usually <20% from baseline (20), which is substantially less than the 49% reduction in severity and 50% reduction in frequency of RP episodes observed for losartan in the present study. That the clinical benefit of losartan was less evident in scleroderma patients is in keeping with the results from other studies which have suggested that treatment with vasodilators is less effective for secondary RP (8,21,22). This may reflect underlying changes in endothelium with its activation and damage, as well as coexistent inflammatory processes (23,24).

Although several studies have reported the effectiveness of nifedipine (4–7), different individual re-

sponses to this drug have been observed (3,21). The 18% overall improvement observed in the nifedipine-treated arm of the study is below that reported in some other trials. A poor response to nifedipine in our study might be attributable to the relatively low dose (40 mg/day), which was selected to minimize side effect–related withdrawals from the control group. Maintenance doses employed for treating refractory RP often exceed 60 mg/day (4,6) but generally require a dose-escalating regimen (6,24). Furthermore, nifedipine has been found to be less effective (24) or of no benefit (8,22) in secondary RP, and this was also our finding.

The results for markers of endothelial cell activation, vascular damage, and extracellular matrix turnover are intriguing but must be interpreted cautiously. It is possible that some of these changes occurred by chance, especially since a number of potentially linked variables were measured. Also, although a number of these markers have been found to be increased in patients with SSc or primary RP (19,25,26), their reliability as disease indicators has not yet been unambiguously demonstrated (27,28). A wide range of values for these markers in this study and in others (19,27) makes the interpretation of changes in serum levels difficult. Disease subsets in the SSc group (29–31), baseline disease activity (27,28,31), and reported circadian variation in the serum levels of some markers (32,33) should be also taken into account.

Nevertheless, several studies have suggested that altered adhesion molecule expression and increased fibrinolysis may be involved in the pathogenesis of both SSc and primary RP (19,27–29). Elevated plasma levels of ET-1, a potent vasoconstrictor, have also been reported in patients with SSc (30,31) as well as in patients with RP (26), and may be a predictor of prognosis in SSc (26,28). In our study, we found elevated baseline levels of circulating ICAM-1, VCAM-1, E-selectin, and ET-1 in SSc, which is in keeping with findings of other groups of investigators (26,28). Losartan treatment was associated with a significant decrease in the levels of VCAM-1 and a large, although not significant, reduction in ICAM-1 and ET-1. This effect of losartan has been reported previously (34) in patients with systemic hypertension.

Profibrotic activity for angiotensin II has been widely described. In particular, angiotensin II can induce collagen and fibronectin synthesis (35,36) and stimulate transforming growth factor β gene expression in fibroblasts and endothelial cells (37,38), thereby promoting extracellular matrix deposition. Losartan has been shown to reduce lung fibroblast proliferation and collagen production induced by angiotensin II in vitro (39). The observed reduction in serum levels of PINP among patients in our study, reflecting the down-regulation of collagen synthesis, was statistically significant in the losartan group. Serum markers of collagen turnover may reflect fibrotic disease activity (40,41), raising the possibility that losartan might have additional disease-modifying potential in SSc, although such a conclusion would be premature from the current study.

Previous attempts to derive reliable, objective, noninvasive measures of blood flow for use in drug studies in RP have had limited success, and our data confirm this, despite significant changes in symptoms of vasospasm. In earlier studies, thermography has been used to quantify dynamic response to drug treatment (42), but its effectiveness has not been widely accepted because of the low reproducibility of the results (17,43). LDF has also been used in interventional studies, with variable outcome (17,44).

In conclusion, the results of this pilot study suggest that 12 weeks of treatment with losartan improves symptoms of vasospasm in RP patients and to a lesser extent in SSc patients. The ability of losartan to modify some serum markers of vascular damage and connective tissue turnover is consistent with the hypothesis that it might also favorably modulate some underlying processes in SSc and RP. We believe that further evaluation in larger studies is warranted, including perhaps examination of its effect on organ-based complications such as pulmonary hypertension and pulmonary fibrosis.

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REFERENCES

1. Raynaud M. On local asphyxia and symmetrical gangrene of the extremities, 1864. London: The New Sydenham Society; 1888.
2. Dowd P, Burnstock G, Marshall I, Bull HA, Foreman J, Goldsmith P. Raynaud's phenomenon. Lancet 1996;346:283–90.
3. Smith CD, McKendry RJ. Controlled trial of nifedipine in the treatment of Raynaud's phenomenon. Lancet 1982;2:1299–301.
4. Nilsson H, Jonason T, Leppert J, Ringqvist I. The effect of the calcium-entry blocker nifedipine on cold-induced digital vasospasm: a double-blind crossover study versus placebo. Acta Med Scand 1987;221:53–60.
5. Gush RJ, Taylor LJ, Jayson MI. Acute effects of sublingual nifedipine in patients with Raynaud's phenomenon. J Cardiovasc Pharmacol 1987;9:628–31.
6. Gjorup T, Kelbaek H, Hartling OJ, Nielsen SL. Controlled double-blinded trial of the clinical effect of nifedipine in the

treatment of idiopathic Raynaud's phenomenon. Am Heart J 1986;111:742–5.
7. Kallenberg CGM, Wouda AA, Kuitert J, Tijssen J, Wesseling H. Nifedipine in Raynaud's phenomenon: relationship between immediate, short term and long term effects. J Rheumatol 1987;14: 284–90.
8. Meyrick Thomas RH, Rademaker M, Grimes SM, Mackay A, Kovacs IB, Cook ED, et al. Nifedipine in the treatment of Raynaud's phenomenon in patients with systemic sclerosis. Br J Dermatol 1987;117:237–41.
9. Weber A, Bounameaux H. Effects of low-dose nifedipine on a cold provocation test in patients with Raynaud's disease. J Cardiovasc Pharmacol 1990;15:853–5.
10. Sarkozi J, Bookman A, Mahon W, Ramsay C, Detsky AS, Keystone EC. Nifedipine in the treatment of idiopathic Raynaud's syndrome. J Rheumatol 1986;13:331–6.
11. Timmermans P, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, et al. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev 1993;45:205–51.
12. Johnston CI. Angiotensin receptor antagonist: focus on losartan. Lancet 1995;346:1403–7.
13. Elliott HL. Angiotensin II antagonists: efficacy, duration of action, comparison with other drugs. J Hum Hypertens 1998;12:271–4.
14. Challenor VF. Angiotensin converting enzyme inhibitors in Raynaud's phenomenon. Drugs 1994;48:864–7.
15. Pancera P, Sansone S, Secchi S, Covi G, Lechi A. The effects of thromboxane A_2 inhibition (picotamide) and angiotensin II receptor blockade (losartan) in primary Raynaud's phenomenon. J Intern Med 1997;242:373–6.
16. Caskey FJ, Thacker EJ, Johnston PA, Barnes JN. Failure of losartan to control blood pressure in scleroderma renal crisis [letter]. Lancet 1997;349:620.
17. Herrick AL, Clark S. Quantifying digital vascular disease in patients with primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis 1998;57:70–8.
18. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
19. Herrick AL, Illingworth K, Blann A, Hay CR, Hollis S, Jayson MI. Von Willebrand factor, thrombomodulin, thromboxane, β-thromboglobulin and markers of fibrinolysis in primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis 1996;55:122–7.
20. Black CM, Halkier-Sorensen L, Belch JJ, Ullman S, Madhok R, Smit AJ, et al. Oral iloprost in Raynaud's phenomenon secondary to systemic sclerosis: a multicentre, placebo-controlled, dose-comparison study. Br J Rheumatol 1998;37:952–60.
21. Roedeheffer RJ, Rommer JA, Wigley F, Smith CR. Controlled double blind trial of nifedipine in the treatment of Raynaud's phenomenon. N Engl J Med 1983;308:880–3.
22. Kirch W, Linder HR, Hutt HJ, Ohnhaus EE, Mahler F. Ketanserin versus nifedipine in secondary Raynaud's phenomenon. Vasa 1987;16:77–80.
23. Matucci-Cerinic M, Generini S, Pignone A. New approaches to the treatment of Raynaud's phenomenon. Curr Opin Rheumatol 1997;9:544–56.
24. Belch J, Ho M. Pharmacotherapy of Raynaud's phenomenon. Drugs 1996;52:682–95.
25. Blann AD, Herrick AL, Jayson MI. Altered levels of soluble adhesion molecules in rheumatoid arthritis, vasculitis and systemic sclerosis. Br J Rheumatol 1995;34:814–9.
26. Yamane K. Endothelin and collagen vascular disease: a review with special reference to Raynaud's phenomenon and systemic sclerosis. Intern Med 1994;33:579–82.
27. Denton CP, Bickerstaff MCM, Shiwen X, Carulli MT, Haskard DO, Dubois RM, et al. Serial circulating adhesion molecule levels reflect disease severity in systemic sclerosis. Br J Rheumatol 1995,34:1048–54.
28. Gruschwitz MS, Hornstein OP, von den Driesch P. 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.
29. Veale DJ, Kirk G, McLaren M, Belch JJF. Clinical implications of soluble intercellular adhesion molecule-1 levels in systemic sclerosis. Br J Rheumatol 1998;37:1227–8.
30. Yamane K, Miyauchi T, Suzuki N, Yuhara T, Akama T, Suzuki H, et al. Significance of plasma endothelin-1 levels in patients with systemic sclerosis. J Rheumatol 1992;19:1566–71.
31. Vancheeswaran R, Magoulas T, Efrat G, Wheeler-Jones C, Olsen I, Penny R, et al. Circulating endothelin-1 levels in systemic sclerosis subsets—a marker of fibrosis or vascular dysfunction? J Rheumatol 1994;21:1838–44.
32. Maeda M, Kachi H, Kitajima Y. Circadian variation of blood coagulation/fibrinolysis molecular markers in progressive systemic sclerosis (PSS). J Dermatol Sci 1996;13:18–24.
33. Maeda M, Kachi H, Takagi H, Kitajima Y. Is there circadian variation of plasma endothelin (ET-1) in patients with systemic sclerosis (SSc)? J Dermatol Sci 1997;16:38–44.
34. D'Uscio LV, Shaw S, Barton M, Luscher TF. Losartan but not verapamil inhibits angiotensin II-induced tissue endothelin-1 increase: role of blood pressure and endothelial function. Hypertension 1998;31:1305–10.
35. Crawford DC, Chobanian AV, Brecher P. Angiotensin II induces fibronectin expression associated with cardiac fibrosis in the rat. Circ Res 1994;74:727–39.
36. Ju H, Zhao S, Jassal D, Dixon IM. Effect of AT1 receptor blockade on cardiac collagen remodelling after myocardial infarction. Cardiovasc Res 1997;35:223–32.
37. Chua C, Diglio C, Siu B, Chua BH. Angiotensin II induces TGF-β1 production in rat heart endothelial cells. Biochim Biophys Acta 1994;1223:141–7.
38. Junaid A, Rosenberg ME, Hostetter TH. Interaction of angiotensin II and TGF-β in the rat remnant kidney. J Am Soc Nephrol 1997;8:1732–8.
39. Marshall RP, McNulty RJ, Laurent GJ. The pathogenesis of pulmonary fibrosis: is there a fibrosis gene? Int J Biochem Cell Biol 1997;29:107–20.
40. Hunzelmann N, Risteli J, Risteli L, Sacher C, Vancheeswaran R, Black CM, et al. Circulating type I collagen degradation products: a new serum marker for clinical severity in patients with scleroderma? Br J Dermatol 1998;139:1020–5.
41. Kikuchi K, Ihn H, Sato S, Igarashi A, Soma Y, Ishibashi Y. Serum concentration of procollagen type 1 carboxyterminal propeptide in systemic sclerosis. Arch Dermatol Res 1994;286:77–80.
42. Hahn M, Klyszcz T, Junger M, Rassner G. Local cold exposure test as therapy control in patients with Raynaud's phenomenon: comparison between laser Doppler fluxmetry and simultaneous red blood cell velocity measurements in nailfold capillaries. Br J Dermatol 1995;133:704–9.
43. Watanabe I, Sagawa A, Baba Y, Atsumi T, Jodo S, Amazaki Y, et al. Thermography of collagen diseases with Raynaud's phenomenon. Ryumachi 1991;31:167–74.
44. Rademaker M, Cooke ED, Almond NE, Beacham JA, Smith RE, Mant TGK, et al. Comparison of intravenous infusions of iloprost and oral nifedipine in treatment of Raynaud's phenomenon in patients with systemic sclerosis: a double blind randomized study. BMJ 1989;298:561–4.