Statins: Potentially Useful in Therapy of Systemic Sclerosis-related Raynaud's Phenomenon and Digital Ulcers

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ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is characterized by fibrosis and widespread vascular pathology. Raynaud's phenomenon (RP) and digital ulceration are prominent manifestations of vascular involvement in SSc. Digital ulcers (DU) remain a serious complication, and effective therapy remains elusive. Statins display pleiotropic effects on endothelial function that may potentially retard vascular injury. We evaluated the potential efficacy of statin therapy in endothelial dysfunction and in management of RP and DU.

Methods. Eighty-four SSc patients who fulfilled the American College of Rheumatology criteria for classification of SSc with secondary RP despite ongoing vasodilator therapy were randomized into 2 groups; the first group (n = 56) received 40 mg/day atorvastatin for 4 months; the second group (n = 28) received placebo. Seventy-five healthy volunteers served as controls. Assessment of RP and DU was performed monthly. The primary outcome measure was the number of DU. Secondary endpoints included the modified Scleroderma Health Assessment Questionnaire Disability Index (SHAQ-DI), safety, and tolerability. Measurement of functional status in relation to RP included the SHAQ-DI, visual analog scale (VAS) for RP, DU and pain scales, and VAS for physician's global assessment for health. Endothelial damage markers were also assessed. Endothelium-dependent flow-mediated dilatation was measured by high-resolution echo-Doppler ultrasonography.

Results. The overall number of DU was significantly reduced in the statin group. Among patients in the statin group a mean of 1.6 new ulcers developed per patient compared to 2.5 new ulcers per patient in the placebo group. There was a statistically significant improvement in SHAQ-DI score in patients receiving statin versus those on placebo. VAS for RP, DU severity, and pain scales and the VAS for physician global assessment improved significantly in the statin group compared to the placebo group. Endothelial markers of activation showed statistically significant improvement from baseline values in the statin versus the placebo group.

Conclusion. Our results showed that statins retarded vascular injury and improved patient function. The findings suggest that statins may aid in treating RP and DU in SSc patients. Given the safety and relative low cost of statins and good patient tolerability to this class of drugs, statins may be of clinical benefit in SSc patients. (First Release Aug 15 2008; J Rheumatol 2008;35:1801–8)

Key Indexing Terms:
SYSTEMIC SCLEROSIS DIGITAL ULCERS RAYNAUD'S PHENOMENON STATINS

Systemic sclerosis (SSc) is a chronic connective tissue disorder that has been considered one of the most difficult to treat and most challenging of the rheumatic disorders, due to its multiorgan manifestations, severity, and unpredictable course and outcome.

The etiology of SSc is unknown; it is characterized by excessive cutaneous and visceral fibrosis, an aberrant immune activation, and widespread, pronounced alterations in the microvasculature with structural and functional vasculopathy[1,2].

The vascular endothelium represents the main regulatory component of the vessel wall. Endothelial dysfunction and structural wall abnormalities are early manifestations of many vascular disorders including SSc-related vascular disease. Further, endothelial activation and damage are primary events throughout the course of the disease[3].

The pathogenesis of the structural vascular abnormalities in SSc is believed to involve endothelial cell apoptosis, upregulation of adhesion molecules, and the interplay of a large number of cytokines and growth factors and pericyte activation[3,4].

Evidence indicates that vascular injury is enhanced in SSc. The proposed mechanisms of vascular injury include ischemia-reperfusion reaction, platelet activation, inflam-

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matory-immune response, oxidative stress, and an imbalance between coagulation and fibrinolysis[5,6]. The inherent damage to the endothelial cells leads to an increased ratio of endothelial-derived vasoconstricting agents, such as endothelin-1 (ET-1) and impaired endothelial-derived vasodilating agents such as nitric oxide (NO)[7].

The exact mechanisms of the factors that induce endothelial dysfunction are unclear; there are several serological biomarkers that reflect the vasculopathy of the disease including proinflammatory cytokines, chemokines [monocyte chemotactic protein-1 (MCP-1)], cell adhesion molecules, and markers of oxidant injury as well as mediators such as ET-1 and NO.

The earliest and most evident manifestation of the vascular involvement in SSc is Raynaud's phenomenon (RP), followed by microvascular damage that can progress to digital ulceration, an extremely painful, debilitating, and visible manifestation. Digital ulcers (DU) represent a major clinical problem in SSc, occurring in up to 50% of patients with limited or diffuse SSc[8]. DU are estimated to occur within 4 years of diagnosis in 65% of patients with SSc, causing local pain and substantial functional impairment and disability[9]. Development of DU can significantly affect a patient's quality of life[8-10].

Therapeutic management of SSc-related RP and DU remains a challenge. Effective therapy remains elusive, and few agents have shown vascular remodeling effects. Therapeutic agents that could potentially modify the vasculopathy are thus urgently needed. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) display numerous effects often independent of the well established lipid-lowering effects that may be of benefit in retarding or preventing vascular injury and ischemic vascular events[11]. Extensive research suggests that the clinical benefits of these drugs could be related to an improvement in endothelial dysfunction, a reduction in blood thrombogenicity, anti-inflammatory properties, and, recently, immunomodulatory actions[12].

These pleiotropic effects of statins revealed in experimental and clinical trials suggest that statins may modify the progression of vascular injury[11,12]. To date, only a very limited number of studies have evaluated the clinical benefits of statins on patients with SSc[13,14]. Accordingly, our aim was to evaluate the potential efficacy of statin therapy in the amelioration of endothelial dysfunction and in the management of RP and digital ulceration in patients with SSc.

MATERIALS AND METHODS

Study design. This was a randomized, double-blind, placebo-controlled study undertaken at our institution. Recruitment was done during the cold winter months, December to March. All subjects gave informed consent, and the study was approved by the Ethics Committee of our institution. Following informed consent, eligibility criteria and clinical status were assessed at the first visit.

Participants. Initially, 140 patients were screened to assemble the study population. Eighty-four SSc patients were selected (68 women, 16 men), mean age 48.6 ± 9.4 years, mean disease duration 6.7 ± 8.6 years (beginning when the first non-Raynaud's symptom occurred), and mean duration of RP 8.0 ± 7.2 years. All patients fulfilled the 1980 American College of Rheumatology criteria for the classification of SSc[15]. Diffuse and limited SSc were defined according to the criteria of LeRoy, *et al*[16].

Inclusion criteria included RP and history of a documented DU secondary to SSc within the last 12 months despite ongoing vasodilator therapy. Exclusion criteria included smoking, diabetes mellitus, hypercholesterolemia, hypertension, cardiac insufficiency, coexisting hepatic and renal diseases, and use of drugs known to interact with statins. Patients were matched for age, sex, SSc classification, severity, and concomitant medications. Seventy-five healthy age and sex matched volunteers recruited from hospital staff and visitors served as controls. The purpose of the control group was to provide normal baseline values for inflammatory markers.

Randomization and blinding. All subjects and physicians were blinded to group assignment and treatment allocation. Patients were randomized into 2 parallel groups using a 2:1 atorvastatin to placebo ratio and were assigned 4 months' treatment. This method of randomization was selected to optimize recruitment and to increase the number of patients receiving therapy. Informed consent was obtained from all participants prior to their enrollment into the study.

Assessment and treatment protocol. Systemic assessment was done as recommended[17]. Skin thickness was quantified using the modified Rodnan skin score, on a scale of 0–51[18]. Following a thorough baseline clinical evaluation, patients were randomized into groups. The first group consisted of 56 patients, assigned to receive 40 mg/day of atorvastatin for 4 months, and the second group consisted of 28 patients who were given identical placebo tablets for 4 months. The dosage of 40 mg atorvastatin was chosen based on experience from preceding studies, and because it is a hydrophobic statin having the ability to enter endothelial cells. Both groups were allowed to continue their existing therapy (vasodilators including calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, low-dose corticosteroids, antiplatelet agents (aspirin), and proton pump inhibitors) provided the dosages were kept constant. No patient was taking immunosuppressants or endothelin receptor antagonists at the time of the study.

DU assessment including number of new and existing ulcers was scored, and measurement of functional status in relation to RP was done using the modified Scleroderma Health Assessment Questionnaire (SHAQ), which included the HAQ Disability Index (HAQ-DI; scale 0–3)[19] at each monthly visit. Ulcers were defined as loss of surface epithelium. We scored and photographed ulcers at or distal to the proximal interphalangeal joint only. The longest diameter of ulcers was measured. Only direct ulcer measurements were scored; photographs were not used in scoring, as photographic data are difficult to interpret.

Visual analog scales (VAS) for RP and DU severity, pain, and overall disease severity (scale 0–10) and VAS for physician's global assessment for health (scale 0–10) were scored at each monthly visit. The HAQ measures physical disability in 8 domains of activity. The use of aids and devices was not taken into account. The SHAQ includes the disability and pain scale of the HAQ plus scleroderma-specific VAS scaled 0–10[20]. Biomarkers of endothelial injury including ET-1, circulating NO levels, intercellular adhesion molecule-1 (ICAM-1), soluble E-selectin (sE-selectin), and the chemokine monocyte chemoattractant protein-1 (MCP-1) were evaluated using specific ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Thrombomodulin (Dako, Glostrup, Denmark) and von Willebrand factor (Stago, Asnières, France) concentrations were measured using commercial kits. Concentrations of the proinflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured in serum samples with commercial sandwich ELISA kits (Quantikine, R&D Systems) following the manufacturer's instructions. Fibrinogen (Fibri-test), high sensitivity C-reactive protein (hsCRP; ELISA), erythrocyte sedimentation rate (ESR;

Westergren method), lipid peroxide, and malonylaldehyde (MDA) levels (calorimetric method) were also assessed in SSc patients and compared to 75 healthy controls at baseline. The results were interpolated from the standard reference curve provided with each kit.

The effect of atorvastatin on peripheral macrovascular function was assessed by endothelium-dependent dilatation of the brachial artery. Endothelium-dependent flow-mediated dilatation (FMD) induced by 5 minutes of forearm ischemia was measured as the percentage of change from baseline (FMD%) by high-resolution echo-Doppler ultrasonography in patients and controls. Patients were studied at baseline and after 4 months of therapy. Safety was assessed by monthly clinical and laboratory monitoring.

Study endpoints and outcome measures. The primary outcome measure was the number of new DU occurring during the study period. Secondary endpoints included the SHAQ-DI, the Scleroderma HAQ subsets for dressing/grooming, eating and hand grip, and assessment of safety and tolerability of the atorvastatin therapy.

Statistical methods. Collection of data and statistical analyses were performed blind to treatment allocation. All patients were included in all analyses on an intention-to-treat basis. Statistical significance was based on a 5% level. Descriptive data are presented as the mean ± standard deviation (SD) or as number and percentage. Data were analyzed with the following nonparametric statistical methods: Kruskal-Wallis analysis of covariance and Mann-Whitney and Wilcoxon tests. Differences between treatments in terms of baseline to 4-month change were assessed by the use of analysis of covariance. Variables found to be significantly associated ($p < 0.05$) by univariate analysis were entered into a multivariate logistic regression analysis.

RESULTS

Table 1 summarizes the baseline characteristics of the study group. The patients were similar in age, sex, SSc subset, mean disease duration, mean duration of RP, and disease severity. The antibodies detected at the time of investigation included anticentromere antibodies found in 62 of the 66 patients with lcSSc, antitopoisomerase antibodies detected in 16 of the 18 patients with dsSSc, and antinuclear antibodies detected in 63 of the 84 patients. Total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol levels were similar in all patients. The concomitant therapy of the atorvastatin-treated and placebo-treated groups is shown in Table 1.

The disease characteristics at baseline and after 4 months of therapy are illustrated in Table 2. At least one DU was found in 53.5% of patients at entry. The mean number of ulcers per patient at entry was similar in the 2 treatment groups. Scores for patients' assessments of Raynaud's severity, ulcer severity, ulcer pain, and SSc severity at baseline were similar in both treatment groups. The severity scores for gastrointestinal disease, mean ± SD 2.8 ± 3.8, for respiratory function disease, 2.8 ± 2.7, and for severity of scleroderma, 5.9 ± 4.5, were similar in both treatment groups. The overall SHAQ-DI was also similar in the atorvastatin and

Table 1. Characteristics of the study population at baseline. Values are given as mean (SD) or numbers (percentage).

Characteristic	All Patients, n = 84	Atorvastatin, n = 56	Placebo, n = 28
Age, yrs	48.6 ± 9.4	48.5 ± 9.5	48.8 ± 9.6
Female/male	68/16	46/10	20/8
Subset			
Limited cutaneous SSc	61	41	20
Diffuse cutaneous SSc	23	15	8
Mean disease duration, yrs	6.7 ± 8.6	6.8 ± 8.5	6.6 ± 8.8
Mean duration of RP, yrs	8.0 ± 7.2	8.1 ± 7.0	7.9 ± 7.3
Modified Rodnan skin score (scale 0-51)	12 ± 1.7 (6-36)	11 ± 1.9 (6-37)	13 ± 1.3 (7-37)
Organ involvement (%)			
Lung fibrosis	25 (30)	16 (29)	9 (32)
PAH	30 (36)	20 (36)	10 (36)
Esophagus	59 (70)	39 (70)	20 (71)
Kidney	9 (11)	6 (11)	3 (11)
Joints	19 (23)	12 (21)	7 (25)
Heart	8 (10)	5 (9)	3 (11)
Disease activity score, mean ± SD	2.5 ± 1.6	2.4 ± 1.8	2.5 ± 1.7
Concomitant treatment (%)			
Vasodilators			
Calcium channel blockers	73 (87)	48 (86)	25 (89)
ACE inhibitors	40 (48)	26 (46)	14 (50)
Angiotensin receptor blockers	18 (21)	12 (21)	6 (21)
Low-dose aspirin	40 (48)	27 (48)	13 (46)
Pentoxyphylline	37 (44)	23 (41)	14 (50)
H_2 blockers	38 (45)	23 (41)	15 (54)
Proton pump inhibitors	34 (40)	22 (39)	12 (43)
Low-dose corticosteroids (< 7.5 mg/day)	24 (29)	16 (29)	8 (29)

RP: Raynaud's phenomenon, PAH: pulmonary artery hypertension, ACE: angiotensin-converting enzyme.

Table 2. Change from baseline after 4 months' therapy — effect on digital ulcers (DU) and Raynaud's phenomenon (RP). Values are given as mean (SD).

Characteristic	Atorvastatin	Placebo	Δ, %	p
No. of DU				
Baseline	3.3 (1.3)	3.4 (1.2)		
4 months	2.4 (0.9)	3.0 (1.3)	–26	0.001
Mean number of new DU/patient	1.6	2.5	–36	0.003
VAS (0–10)				
RP severity				
Baseline	6.0 (3.3)	6.0 (3.4)		
4 months	4.9 (3.5)	5.7 (3.8)	–18	0.005
Ulcer severity				
Baseline	6.0 (3.5)	6.1 (3.2)		
4 months	4.9 (4.1)	5.7 (3.4)	–18	0.001
Ulcer pain				
Baseline	5.9 (3.0)	6.0 (3.5)		
4 months	4.7 (2.7)	5.8 (4.6)	–20	0.004
Physician global assessment				
Baseline	6.4 (4.8)	6.3 (4.7)		
4 months	5.1 (5.0)	5.9 (4.9)	–21	0.003

Δ: change at the end of study period.

placebo-treated groups. SHAQ scores for dressing/grooming, eating, and hand grip were similar in both treatment arms. The primary outcome measure was the number of new DU occurring during the treatment period. There was a significant reduction in the overall number of DU in patients receiving atorvastatin and a significant reduction in the mean number of new DU per patient, with a mean of 1.6 new ulcers per patient compared to 2.5 new ulcers per patient among those receiving placebo (Table 2). The absolute number of true responders was 14 patients. Atorvastatin therapy showed similar improvements in both subsets of SSc patients, both the lcSSc and the dcSSc groups (data not shown).

A statistically significant decrease of the VAS scores for RP, DU severity and pain, and overall disease severity between the baseline period and posttreatment period in the atorvastatin-treated group compared to the placebo group was found (Table 2). There was also a significant change in the physician global assessment in the atorvastatin-treated group compared to the placebo group. The minimal improvement of the HAQ-DI score observed in patients receiving atorvastatin was statistically and clinically significant (Table 3). There was a statistically significant improvement in scores for dressing/grooming, eating, and hand grip in patients receiving atorvastatin compared to those on placebo (Table 3).

The circulating levels of endothelial activation/injury markers were measured before and after atorvastatin treatment. Data before atorvastatin treatment were compared between the patient groups and the healthy control group. All markers were significantly higher at baseline compared to normal healthy controls. NO levels at baseline, however,

Table 3. Change from baseline after 4 months' therapy — effect on Scleroderma Health Assessment Questionnaire Disability Index (SHAQ-DI). Values are given as mean (SD).

Characteristic	Atorvastatin	Placebo	Δ,%	p
SHAQ-DI				
Baseline	0.99 (0.63)	0.96 (0.64)		
4 months	0.80 (0.59)	0.96 (0.51)	–20	0.001
Dressing/grooming				
Baseline	0.98 (0.79)	0.98 (0.80)		
4 months	0.80 (0.9)	1.18 (1.1)	–18	0.001
Eating				
Baseline	0.99 (0.66)	0.98 (0.67)		
4 months	0.88 (0.75)	1.12 (0.71)	–11	0.05
Hand grip				
Baseline	1.00 (0.88)	0.99 (0.87)		
4 months	0.80 (0.85)	1.11 (0.88)	–20	0.001

Δ: change at the end of study period.

were significantly lower in patients compared to healthy controls. Prior to treatment there were no differences in levels of inflammatory markers between the atorvastatin-treated group and the placebo group. At 4 months, mean levels of the inflammatory markers IL-6, TNF-α, ESR and hsCRP, the endothelial activation markers ICAM, sE-selectin, ET-1 and MCP, and the oxidative markers lipid peroxide and MDA, as well as vWF activity and fibrinogen decreased significantly in the atorvastatin group compared to the placebo group (Table 4). At 4 months, the mean level of NO was significantly increased in the atorvastatin-treated group compared to the placebo arm.

The percentage FMD was significantly lower in all SSc patients compared to controls (3.66 ± 2.98% vs 8.4 ± 3.72%, respectively) at baseline and increased significantly in the atorvastatin-treated group at 4 months compared to the placebo group (5.3 ± 2.89% vs 3.5 ± 2.42%; Figure 1).

As expected, the total cholesterol level in the atorvastatin-treated group was significantly reduced compared to baseline values (reduction of 21% at 16 weeks); however, no

Table 4. Mean levels of biomarkers of endothelial injury in the study population. Data are mean (SD).

Marker	Atorvastatin, n = 56	Placebo, n = 28	Controls, n = 75
IL-6, pg/ml			
Baseline	26.1 (11.5)*	25.5 (11.8)*	2.6 (1.9)
4 months	19.4 (12.1)**	25.8 (11.5)	
TNF-α, pg/ml			
Baseline	20.9 (9.5)*	19.9 (9.1)*	9.7 (5.8)
4 months	14.5 (8.8)**	20.1 (9.6)	
ET-1, pg/ml			
Baseline	3.2 (1.7)*	2.98 (1.9)*	1.2 (0.3)
4 months	2.4 (1.2)**	3.0 (1.5)	
NO, µmol/L			
Baseline	24.8 (4.5)*	24.1 (4.8)*	32.3 (4.9)
4 months	30.4 (4.4)**	23.9 (4.5)	
TM, ng/ml			
Baseline	48.5 (8.3)*	48.9 (8.0)*	23.6 (7.6)
4 months	40.7 (8.1)**	49.4 (8.8)	
Soluble E-selectin, pg/ml			
Baseline	39.4 (8.9)*	39.9 (8.3)*	26.8 (4.6)
4 months	31.4 (8.5)**	41.1 (9.2)	
vWF, pg/ml			
Baseline	229.4 (27.9)*	228.8 (28.9)*	126.6 (18.7)
4 months	202.3 (26.6)**	230.4 (26.9)	
ICAM-1, pg/ml			
Baseline	315.4 (69.5)*	313.7 (69.8)*	188.4 (25.1)
4 months	297.7 (66.7)*	319.3 (68.5)	
MCP-1, pg/ml			
Baseline	215 (94.3)*	217 (92.7)*	125.5 (43.7)
4 months	198 (90.1)**	216 (92.5)	
Fibrinogen, mg/dl			
Baseline	370.2 (68.5)*	367.9 (68.2)*	186 (33.5)
4 months	328.6 (67.9)**	375.8 (68.8)	
hsCRP, mg/l			
Baseline	3.79 (1.8)*	3.85 (1.4)*	0.6 (0.3)
4 months	3.14 (1.5)**	3.91 (1.5)	
ESR, mm/h			
Baseline	29.5 (4.5)*	27.5 (4.9)*	14 (1.6)
6 months	21.5 (4.0)**	25.4 (5.0)	
Lipid peroxide, µmol/l			
Baseline	5.92 (2.5)*	5.88 (2.2)*	2.35 (1.5)
4 months	4.80 (2.2)**	5.99 (2.7)	
MDA, µmol/l			
Baseline	5.95 (3.7)*	5.99 (3.5)*	2.63 (1.7)
4 months	4.91 (3.5)**	6.12 (3.3)	

* Significantly different from control group; **significantly different from placebo group. IL-6: interleukin 6; TNF-α: tumor necrosis factor-α; ET-1: endothelin-1; NO: nitric oxide; TM: thrombomodulin; vWF: von Willebrand factor; ICAM-1: intercellular adhesion molecule-1; MCP: monocyte chemoattractant; hsCRP: high sensitivity C-reactive protein; ESR: erythrocyte sedimentation rate.

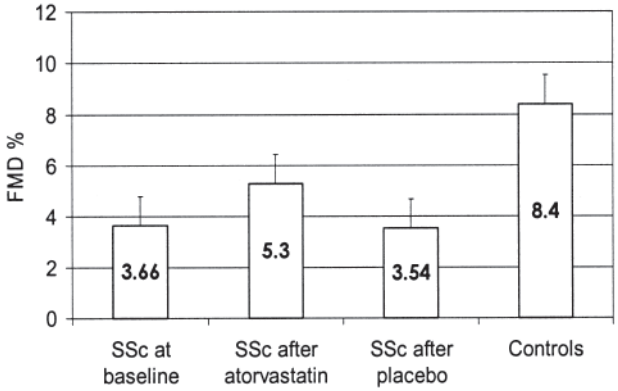

Figure 1. Flow-mediated vasodilatation (FMD, %) in patients with systemic sclerosis (SSc) at baseline and after therapy compared to healthy controls.

measurement dropped to levels requiring cessation of the drug.

No patient receiving atorvastatin experienced any serious adverse events necessitating discontinuation of therapy and there were no dropouts.

DISCUSSION

Abnormalities of vascular structure and function are well recognized in SSc. Vascular alterations occur early in the pathogenesis and contribute to the morbidity and mortality of this disease. Digital ulcers are an important complication of SSc, having profound and sustained effects on the quality of life of a large number of SSc patients. To date, effective therapy for RP and DU is mostly disappointing and elusive. The beneficial effect of statins against various types of vascular diseases has led several investigators to propose them as a potential treatment for SSc vasculopathy[21].

We observed a significant reduction in the number of DU in the atorvastatin-treated group compared to those receiving placebo. The patients' and physicians' assessments of disease activity for RP, DU, and HAQ-DI (a functional disability index) were significantly improved in the atorvastatin-treated group compared to the placebo-treated group. Similarly, Kuwana, *et al* in an open-label, prospective study reported improvement in the manifestations of SSc-RP during atorvastatin therapy, with significant reduction in RP score and patient's VAS assessment at 12 weeks[13].

The beneficial effects observed during atorvastatin treatment could be because statins target key events in the pathogenesis of DU in SSc[22,23].

Statins have several vasculoprotective actions including vasodilatation, inhibition of platelet aggregation, inhibition of leukocyte chemotaxis and adhesion to the endothelium, downregulation of adhesion molecules on endothelial cells, and enhanced fibrinolytic action as well as antioxidant actions[24]. These actions can influence the pathophysiological mechanisms responsible for vascular injury in SSc; statins may thus have potential clinical benefit in SSc.

Data from our study indicate that atorvastatin administration in patients with SSc resulted in significant improvement in endothelial dysfunction, shown by improvement in the levels of endothelial activation markers. Mean levels of NO increased and mean levels of ET-1 decreased after atorvastatin therapy compared to the placebo-treated group. In SSc the production of NO is downregulated; besides its vasodilating effects NO appears to inhibit platelet aggregation and adhesion, decrease endothelium permeability, decrease local inflammatory cytokine production, and interfere with adhesion of macrophages and leukocytes to endothelium[7]. Evidence has shown that statins are capable of increasing NO production by causing upregulation of endothelial-derived NO synthase (eNOS)[25]. Statins also have effects on the ET-1 pathway by reducing pre-pro-ET-1 mRNA expression and decreasing the expression of endothelin receptors in vascular cells[26]. Statins also improve endothelium-dependent vasorelaxation and increase the number of endothelial progenitor cells[24,27]. Statins may thus improve endothelial function through their effects on NO.

In our study, atorvastatin reduced levels of the adhesion molecules ICAM-1 and sE-selectin and the chemokine MCP-1. Previous studies showed that statins inhibit interactions between leukocytes and endothelial cells that necessarily precede leukocyte migration from the vasculature, and downregulate expression of adhesion molecules and chemokines (MCP-1) and their receptors on endothelial cells, and also downregulate soluble ICAM-1 and E-selectin[28-30]. Statins have been shown to inhibit production of MCP-1 both *in vivo* and *in vitro*[29,30].

Structural changes in SSc are characteristic of a proliferative occlusive vasculopathy. Narrowing of the vessel lumen and endothelial dysfunction further propagate tissue ischemia by inducing platelet aggregation and thrombosis. Our findings showed that atorvastatin reduced fibrinogen, vWF, and thrombomodulin levels, suggesting an antithrombogenic effect of statins. These results are in agreement with those of Furukawa and colleagues[14].

We observed statins also reduced levels of the inflammatory cytokines IL-6 and TNF-α and the acute-phase reactants ESR and CRP. Several lines of evidence indicate that SSc presents deregulated production of cytokines implicated in vascular damage and fibrosis[29-31]. In SSc, tissue-infiltrating T cells initiate local production of cytokines and growth factors that cause transdifferentiation of tissue-bound fibroblasts and smooth-muscle cells in the vessel wall into myofibroblasts[32]. These statin-mediated improvements in endothelial function may be attributed to their antiinflammatory and immunomodulator properties[33].

Our study also demonstrated that statins reduced levels of the oxidative stress markers lipid peroxide and MDA. Numerous *in vitro* and *in vivo* studies suggest that free-rad-

ical generation is a key event in the pathogenesis of SSc and that oxygen free radicals may initiate endothelial damage and tissue fibrosis[34,35]. Further, free radicals may be generated by ischemia-reperfusion, a characteristic chronic widespread phenomenon in SSc. Statins interfere with the generation of reactive oxygen species by endothelial cells. At least 2 independent mechanisms appear responsible for this effect, the reduction of myeloperoxidase-derived and NO-derived oxidants and inhibition of their expression and activation by the reduced nicotinamide adenine dinucleotide phosphatase oxidation activity[36,37].

In this study, treatment with atorvastatin resulted in significant improvement in percentage FMD (indicating endothelium-dependent vasodilatation), suggesting an effect of statins on peripheral macrovasculature in SSc. This is in agreement with the findings of Szucs, *et al*, who showed that damage of large vessels occurred in a significant proportion of their SSc patients[38].

Our study has gone a step further to confirm and verify that statins help maintain vascular integrity. Based on these considerations, it can be suggested that statins may influence the fundamental mechanism underlying the pathogenesis of SSc. Statins may have the capacity to improve vascular outcome and to reduce the manifestations of RP, particularly if instituted early in the disease process, in the prescleroderma stage.

Data from our study suggest that atorvastatin may exert beneficial effects in SSc by protecting the endothelium and improving its functional activity.

Limitations of our study include the sample size and the cross-sectional design. Further, the longterm vascular effects of statins including effects after discontinuation of therapy were not evaluated.

The observed vascular-modifying effect of statins may be attributed to their pleiotropic actions including improvement of vascular function and suppression of inflammation. We observed statin-mediated improvements in endothelial function of patients with SSc. Given the essential role of statins in preserving vascular structure and function, they may be of clinical benefit in these patients, and may aid in treatment of RP and DU associated with SSc. Larger scale studies are needed in heterogenous populations, as well as within different climates.

REFERENCES

1. Jimenez SA, Derk CT. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. Ann Intern Med 2004;140:37-50.
2. Denton CP, Black CM. Targeted therapy comes of age in scleroderma. Trends Immunol 2005;26:596-602.
3. Kahaleh BM. Vascular involvement in systemic sclerosis. Clin Exp Rheumatol 2004;22 Suppl 33:S19-23.
4. Denton CP, Black CM. Scleroderma—clinical and pathological advances. Best Pract Res Clin Rheumatol 2004;18:271-90.
5. Kahaleh MB, LeRoy EC. Autoimmunity and vascular involvement in systemic sclerosis. Autoimmunity 1999;31:195-214.
6. Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med 1999;340:115-26.
7. De Caterina R, Libby P, Peng HB, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J Clin Invest 1995;96:60-8.
8. Herrick AL. Vascular function in systemic sclerosis. Curr Opin Rheumatol 2000;12:527-33.
9. Denton CP, Korn J. Digital ulceration and critical digital ischaemia in scleroderma. Scleroderma Care Res 2003;1:12-6.
10. Varga J. New hope for systemic sclerosis patients with digital ulcers. Curr Rheumatol Rep 2005;7:127-8.
11. Davignon J. Beneficial cardiovascular pleiotropic effects of statins. Circulation 2004;109 Suppl:III39-43.
12. Sparrow CP, Burton C, Hernandez M, et al. Simvastatin has anti-inflammatory and anti-atherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol 2001;21:115-21.
13. Kuwana M, Kaburaki J, Okazaki Y, et al. Increase in circulating endothelial precursors by atorvastatin in patients with systemic sclerosis. Arthritis Rheum 2006;54:1946-51.
14. Furukawa S, Yasuda S, Amengual O, et al. Protective effect of pravastatin on vascular endothelium in patients with systemic sclerosis: a pilot study. Ann Rheum Dis 2006;65:1118-20.
15. Masi Al, Rodnan GP, Medsger TA Jr, et al. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 2002;48:2410-20.
16. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subset and pathogenesis. J Rheumatol 1988;15:202-5.
17. Valentini G, Medsger TA Jr, Silman AJ, Bombardieri S. Conclusion and identification of the core set of variables to be used in clinical investigations. Clin Exp Rheumatol 2003;21 Suppl 29:S47-8.
18. Kahaleh B, Meyer O, Scorza R. Assessment of vascular involvement. Clin Exp Rheumatol 2003;21 Suppl:S9-14.
19. 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.
20. Merkel PA, Herlyn K, Martin RW, et al. Measuring disease activity and functional status in patients with scleroderma and Raynaud's phenomenon. Arthritis Rheum 2002;46:2410-20.
21. Derk CT, Jimenez SA. Statins and the vasculopathy of systemic sclerosis: potential therapeutic agents? Autoimmun Rev 2006; 5:25-32.
22. Hummers L, Wigley F. Management of Raynaud's phenomenon and digital ischaemic lesions in scleroderma. Rheum Dis Clin North Am 2003;29:293-313.
23. Lau C, McLaren M, Saniabadi A, Belch J. Increased whole blood platelet aggregation in patients with Raynaud's phenomenon with or without systemic sclerosis. Scand J Rheumatol 1993;22:97-101.
24. Sule SD, Wigley FM. Treatment of scleroderma: an update. Expert Opin Investig Drugs 2003;12:471-82.
25. Laufs U, LaFata V, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMGCoA reductase inhibitors. Circulation 1998;97:1129-35.
26. Xu C, Stenman E, Edvinsson L. Reduction of bFGF-induced smooth muscle cell proliferation and endothelin receptor mRNA expression by mevastatin and atorvastatin. Biochem Pharmacol 2002; 64:497-505.
27. Lekakis J, Mavrikakis M, Papamichael C, et al. Short term estrogen administration improves endothelial function in women with systemic sclerosis and Raynaud's phenomenon. Am Heart J 1998;136:905-12.
28. Zapolska-Downar D, Siennicka A, Kaczmarczyk M, et al.

Simvastatin modulates TNF-α induced adhesion molecule expression in human endothelial cells. Life Sci 2004;75:1287-302.
29. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8 and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194-9.
30. Abou-Raya A, Abou-Raya S, Helmii M. Statins as immunomodulators in systemic sclerosis. Ann NY Acad Sci 2007;1110:670-80.
31. Leung BP, Sattar N, Crilly A, et al. A novel antiinflammatory role for simvastatin in inflammatory arthritis. J Immunol 2003; 170:1524-30.
32. Atamas SP. Complex cytokine regulation of tissue fibrosis. Life Sci 2002;72:631-43.
33. Abeles AM, Pillinger MH. Statins as anti-inflammatory and immunomodulatory agents. A future in rheumatology therapy. Arthritis Rheum 2006;54:393-407.
34. Murrell DF. A radical proposal for the pathogenesis of scleroderma. J Am Acad Dermatol 1993;28:78-85.
35. Volpe A, Biasi D, Caramaschi P, et al. Level of F_2-isoprostanes in systemic sclerosis: correlation with clinical features. Rheumatology Oxford 2006;45:314-20.
36. Shishehbor MH, Brennan ML, Aviles RJ, et al. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation 2003;108:426-31.
37. Rueck Schloss U, Galle J, Holtz J, et al. Induction of NAD(P)H oxidase by oxidized low-density lipoprotein in human endothelial cells; antioxidative potential of hydroxymethylglutaryl coenzyme A reductase inhibitor therapy. Circulation 2001;104:1767-72.
38. Szucs G, Timar O, Szekanecz Z, et al. Endothelial dysfunction precedes atherosclerosis in systemic sclerosis – relevance for prevention of vascular complications. Rheumatology Oxford 2007;46:759-62.