Different patterns of endothelial cell activation in renal and pulmonary vascular disease in scleroderma

R.J. STRATTON, J.G. COGHLAN¹, J.D. PEARSON², A. BURNS³, P. SWENY³, D.J. ABRAHAM and C.M. BLACK

From the Department of Academic Rheumatology and Connective Tissue Disease Unit, ¹Department of Cardiology, and ³Department of Renal Medicine, Royal Free Hospital and School of Medicine, London, and ²Vascular Biology Research Centre, Kings College, London

Received 14 April 1998

Summary

Abnormal endothelial cell function is implicated in the development of scleroderma, and in major life-threatening complications of the disease. The nature of the stimulus leading to abnormal endothelial cell function in scleroderma, scleroderma renal crisis, and scleroderma-associated pulmonary hypertension was investigated by measurement of soluble adhesion molecules, shed by activated endothelial cells, in sera from patients with these conditions. In scleroderma renal crisis, mean levels of soluble E-selectin (p<0.05 limited scleroderma, p<0.0001 diffuse scleroderma), sVCAM-1 (soluble vascular cell adhesion molecule-1) (p<0.05 limited scleroderma, p<0.05 diffuse scleroderma), and sICAM-1 (soluble intercellular adhesion molecule-1) (p<0.0001 limited scleroderma, p<0.0001 diffuse scleroderma) were raised, supporting a model of endothelial cell activation in this complication. Evidence for endothelial cell activation in scleroderma-associated pulmonary hypertension was inconsistent, with normal sE-selectin and normal sVCAM-1 in sera from patients with limited scleroderma and pulmonary hypertension. The endothelial cell phenotype in scleroderma-associated pulmonary hypertension may resemble that of unstimulated cells. The pulmonary vascular and renal vascular lesions associated with scleroderma may arise by distinct pathogenic mechanisms.

Introduction

A firm body of evidence supports involvement of vascular endothelial cells in the pathogenesis of the connective tissue disease scleroderma, and in the development of major vascular complications of the disease.¹ Scleroderma renal crisis (SRC), a syndrome of accelerated hypertension and acute renal failure, is characterized by intimal proliferation, medial hyperplasia and obliteration of the lumen of arcuate and interlobular renal arterioles.² Similar changes are seen in the pulmonary arteries of patients with scleroderma-associated pulmonary hypertension, and may develop in the absence of pulmonary fibrosis.³ Only a small minority of patients suffer both renal vascular and pulmonary vascular disease.⁴

When stimulated by inflammatory cytokines such as interleukin-1 (IL-1) or tumour necrosis factor α (TNFα), endothelial cells show enhanced expression of adhesion molecules, and this mechanism appears important in the recruitment of leukocytes to sites of inflammation.⁵ When activated in this way, endothelial cells shed soluble forms of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, by cleavage at a site adjacent to the plasma membrane.⁶ Previous studies show increased serum concentration of soluble adhesion molecules in conditions associated with widespread damage to the vascular endothelium, such as systemic lupus erythematosus, vasculitis and septicaemic shock.⁷ The nature of the stimulus leading to abnormal endothelial cell function in scleroderma is the subject of investigation in the present paper. The levels of soluble forms of E-selectin, ICAM-1 and VCAM-1 are measured in sera from patients with major
Figure 1. Soluble adhesion molecule concentration by patient subgroup. RP, primary Raynaud’s disease; LSSc, limited scleroderma with no vascular complications; LPHT, limited scleroderma with pulmonary hypertension; LSRC, limited scleroderma with SRC; DSSc, diffuse scleroderma; DPHT, diffuse scleroderma with pulmonary hypertension; DSRC, diffuse scleroderma with SRC. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$; boxed area, normal range.
vascular complications of scleroderma, compared with sera from patients with scleroderma and no major vascular complications, and sera from patients with primary Raynaud’s disease.

Methods

Definition of subgroups

Primary Raynaud’s disease was defined as a history of triphasic colour change with no clinical evidence of a connective tissue disease. Scleroderma and its subsets were defined as described by standard published criteria. Pulmonary hypertension was defined by Doppler echocardiogram showing systolic pulmonary artery pressure of >30 mmHg. SRC was defined as an acute deterioration in renal function with two of the following three characteristics: (i) hypertension >160/90 mmHg; (ii) hypertensive retinopathy; (iii) typical changes of scleroderma renal crisis on renal biopsy.

Patients and samples

A total of 57 patients were studied, with multiple serum samples from each giving 244 samples for analysis in the following groups: primary Raynaud’s disease, (12 patients, 51 samples); limited scleroderma without vascular complications, (6 patients, 32 samples); limited scleroderma with pulmonary hypertension, (10 patients, 32 samples); limited scleroderma with SRC (4 patients, 16 samples); diffuse scleroderma without vascular complications (8 patients, 32 samples); diffuse scleroderma with pulmonary hypertension, (6 patients, 24 samples); diffuse scleroderma with SRC, (11 patients, 57 samples).

Clinical assessment of patients

Patients attended at intervals of between 6 and 12 months for clinical assessment. Internal organ involvement was assessed on an annual basis by lung function studies, colour flow Doppler echocardiography, oesophageal scintiscan and creatinine clearance. Blood was taken at each visit and serum stored at −40°C before assay.

Assay for soluble adhesion molecules

Soluble adhesion molecule levels were assayed in serum samples by standard sandwich ELISA kits from R&D. A standard curve was established for each assay plate. Normal ranges were based on analysis of 104 healthy sera by the manufacturers.

Statistical methods

In the first instance, the results of soluble adhesion molecule concentrations for disease subsets were compared against the normal range by Student’s t
test. Simple visual analysis of serial concentrations of soluble adhesion molecule against time was performed to look for increasing concentration at the time of SRC or with increasingly severe pulmonary hypertension.

Results

Soluble E-selectin

Mean sE-selectin levels were not raised in patients with primary Raynaud’s disease, or limited scleroderma with pulmonary hypertension, (Figure 1a), but were significantly higher in patients with limited scleroderma with no vascular complications, and limited scleroderma complicated by renal crisis. Mean sE-selectin levels were raised in all diffuse scleroderma groups, with the highest levels seen in patients with diffuse scleroderma and pulmonary hypertension.

Soluble VCAM-1

Mean sVCAM-1 levels were raised (nearly twice normal) in both limited and diffuse subsets with SRC (Figure 1b). sVCAM-1 levels were also raised in limited scleroderma with no major vascular complication, but in all other subsets tested the values of sVCAM-1 did not differ from the normal range for healthy individuals.

Soluble ICAM-1

Mean sICAM-1 levels were raised in all groups of patients studied (Figure 1c). In the primary Raynaud’s group, the increase in mean sICAM-1 level was modest (< 25% above normal range) in comparison to the other groups studied. The highest levels of sICAM-1 were seen in patients with diffuse scleroderma complicated by pulmonary hypertension.

Serial measurements of soluble adhesion molecule concentration

Analysis of serial data from patients with primary Raynaud’s, diffuse scleroderma with no vascular complications, and limited scleroderma with no vascular complications, did not show any consistent trend with time. Similarly, results for patients with scleroderma-associated pulmonary hypertension did not show any consistent or sustained increase in soluble adhesion molecule with time. Serial data from patients with diffuse scleroderma complicated by SRC and limited scleroderma complicated by SRC showed no consistent increase in soluble adhesion molecule concentration at the time of SRC.

Discussion

Serious life-threatening complications of the connective tissue disease scleroderma appear to be near-pure vascular disorders related to abnormal endothelial cell function. Post-mortem histopathology studies show arterial lesions characterized by intimal cell proliferation and narrowing of the lumen, but the pathogenesis of these lesions is not understood.

In the present study, the nature of the stimulus leading to abnormal endothelial cell function in major vascular complications of scleroderma has been studied by comparing concentrations of soluble adhesion molecules in sera from patients with vascular complications of the condition, with those from patients with uncomplicated scleroderma, and from patients with primary Raynaud’s disease. Of the adhesion molecules studied, sE-selectin has the greatest specificity for cytokine-activated endothelial cells. Mean sE-selectin concentration was not raised in sera from patients with primary Raynaud’s disease. The mean sE-selectin concentration was moderately raised (around 25% above normal) in sera from patients with limited scleroderma and no evidence of major vascular complications. Of particular interest is the finding of normal mean sE-selectin in the sera from patients with limited scleroderma and pulmonary hypertension, suggesting that in the pulmonary vascular lesions of scleroderma-associated pulmonary hypertension, the endothelial cell phenotype does not resemble that of cytokine-activated cells. By way of contrast, sE-selectin levels were raised (approximately twice normal) in sera from patients with limited scleroderma complicated by SRC. Possible explanations for the disparity between limited scleroderma with pulmonary hypertension and limited scleroderma with SRC include: (i) a mechanism driving the development of the renal vascular lesions distinct from that which leads to pulmonary vascular lesions; (ii) an effect due to the greater hydrostatic pressure in the renal arterioles when compared to pulmonary arterioles; (iii) impaired clearance of sE-selectin in patients with SRC. It seems unlikely that the vascular lesions of SRC and scleroderma-associated pulmonary hypertension arise via completely unrelated mechanisms, but there may be subtle differences which affect the way local endothelial cell function is disturbed. The mechanism by which soluble adhesion molecules are cleared is not known. In view of their molecular mass, 78 kDa in the case of sE-selectin, significant glomerular filtration seems unlikely.

Raised mean sE-selectin was found in all groups
with diffuse scleroderma, and this confirms the findings of a previous study where raised serum sE-selectin concentration was attributed to activation of microvascular endothelial cells in lesional skin.\textsuperscript{11} The highest mean sE-selectin level (more than three times normal) was measured in sera from patients with diffuse scleroderma and pulmonary hypertension. The diffuse scleroderma patients with pulmonary hypertension all had evidence of fibrosing alveolitis (vital capacity of <85% predicted and evidence of fibrosing alveolitis on thoracic CT scan) as a confounding factor, whereas none of the limited scleroderma pulmonary hypertension group had evidence of significant lung fibrosis. One possible explanation for the high sE-selectin seen in diffuse scleroderma with pulmonary hypertension is that inflammation of the pulmonary interstitium leads to activation of the endothelial cells of the pulmonary micro-circulation with increased expression and shedding of sE-selectin, analogous to increased production in lesional skin.

Fibroblasts from patients with scleroderma exhibit enhanced expression and shedding of ICAM-1.\textsuperscript{12,13} Peripheral blood mononuclear cells from scleroderma patients release increased quantities of sICAM-1 \textit{in vitro}.\textsuperscript{14} Previous authors have described elevated sICAM-1 concentrations in patients with scleroderma,\textsuperscript{13–16} and this was confirmed in the present study, where mean sICAM-1 was increased in sera from all groups of scleroderma patients. In the present study, sICAM-1 was raised to approximately the same level in uncomplicated diffuse and uncomplicated limited scleroderma sera, in contrast to a previous study where levels were higher in patients with diffuse disease.\textsuperscript{13} The highest levels of sICAM were measured in sera from patients with diffuse scleroderma and pulmonary hypertension, where mean sICAM-1 was nearly three times normal. As previously described, all of the patients in this subgroup had evidence of fibrosing alveolitis. In a previous study of patients with cryptogenic fibrosing alveolitis, an idiopathic interstitial lung disease, serum sICAM-1 was found to be markedly raised; this was attributed to increased expression of sICAM-1 by pulmonary epithelial cells,\textsuperscript{15} and therefore a similar mechanism could explain the high serum sICAM-1 found in the present study in diffuse scleroderma with pulmonary hypertension.

Mean sVCAM-1 concentration was normal in sera from patients with scleroderma-associated pulmonary hypertension and diffuse scleroderma with no major vascular complications. Mean sVCAM-1 levels were raised in sera from patients with SRC from both diffuse and limited subsets of scleroderma. Levels of sVCAM-1 were raised in sera from the limited scleroderma and no major vascular complication group. In general, there was a disparity between sVCAM-1 concentration and the concentration of the other soluble adhesion molecules studied. In addition to endothelial cells, VCAM-1 expression has been described for stromal fibroblasts in bone marrow, lymphoid dendritic cells and tissue macrophages,\textsuperscript{16} and therefore it is possible that only a minor fraction of serum sVCAM-1 is produced by the vascular endothelial cells. This would explain the lack of concordance between the measurements of sVCAM-1 and other adhesion molecules studied.

Overall, the results suggest that in scleroderma, pulmonary vascular disease can develop without activation of pulmonary endothelial cells, as exemplified by the normal concentration of sE-selectin and VCAM-1 in sera from patients with limited scleroderma and pulmonary hypertension. In contrast, high levels of all three soluble adhesion molecules studied were found in sera from SRC patients, irrespective of the pattern of cutaneous involvement. Therefore in SRC the phenotype of renal artery endothelial cells may resemble that of cytokine-stimulated cells. Endothelial cell function is disturbed in scleroderma, and in major vascular complications of the condition. The stimulus leading to pulmonary vascular disease may be distinct from that affecting the renal vessels in SRC where the endothelial cells appear to be in a highly activated state.

**Acknowledgement**

This work was funded by a grant from the Ernst Scherring Foundation, Berlin.

**References**


