

CLINICAL REPORT

Systemic Sclerosis-related Raynaud's Phenomenon: Effects of Iloprost Infusion Therapy on Serum Cytokine, Growth Factor and Soluble Adhesion Molecule Levels

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Microvascular damage occurs in systemic sclerosis and is associated with increased serum levels of endothelial adhesion molecules and endothelium-associated cytokines, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, endothelin-1 and vascular endothelial growth factor (VEGF). Iloprost, a prostacyclin analogue, induces clinical benefit in patients suffering from scleroderma-related Raynaud's phenomenon. This study was performed to investigate the effect of iloprost infusions on endothelium activation. Serum samples from 12 patients with systemic sclerosis were examined using specific enzyme-linked immunoassays. The serum levels of sICAM-1, sVCAM-1 and soluble E-selectin were initially elevated and significantly reduced after iloprost infusions. The serum concentrations of VEGF and endothelin-1 revealed decreased levels after therapy too. These results indicate that the well-known clinical benefit of iloprost infusions on Raynaud's phenomenon is serologically detectable by a reduction of serum levels of endothelium-associated adhesion molecules, cytokines and growth factors reflecting an improvement in endothelial function. *Key words: ICAM-1; VCAM-1; E-selectin; endothelin-1; VEGF.*

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Systemic sclerosis (scleroderma, SSc) is a chronic progressive disease characterized by excessive production of extracellular matrix by fibroblasts, damage of the endothelium of small vessels with subsequent intimal hyperplasia and tissue ischaemia, and activation of the immune system. Involvement of organs such as the lung, heart, kidneys, gut and skin causes substantial morbidity and mortality.

Since vascular lesions commonly occur early in the course of disease and precede fibrosis, endothelial cells have been implicated in the pathophysiology of fibrosis. Endothelial cell damage is evident in SSc by capillary drop-out and vascular leakage leading to oedema formation. Inflammatory cells will be attracted to damaged sites and migrate to adjacent tissue. In turn, activated endothelial cells will upregulate the expression of adhesion molecules on their cell surface, further recruiting immune cells which release fibroblast activating mediators such as IL-1 and bFGF (1). Previous investigations have shown elevated serum levels of soluble intercellular adhesion molecule 1 (sICAM-1), vascular adhesion molecule 1 (sVCAM 1), E- and P-selectin, and a positive association with *in situ* expression of these molecules in lesional skin and

with clinical disease activity in SSc patients (2–4). Therefore, detection of these parameters might provide a useful tool for characterization and prediction of the disease course and its progression.

Raynaud's phenomenon, a vasospastic disorder triggered by exposure to cold or by emotional stress, is the most common feature of vascular disease in SSc. Unlike patients with primary Raynaud's phenomenon, patients with SSc suffer from both a vasospastic disorder and an underlying vasculopathy (5).

Iloprost, a synthetic stable prostacyclin derivative, is well established in the therapy of peripheral occlusive vascular diseases as well as secondary Raynaud's phenomenon. In SSc-related Raynaud's phenomenon in particular, iloprost has been shown to reduce the number, duration and severity of attacks and to improve acral ischaemic skin ulcers (6).

The aim of our study was to investigate the influence of iloprost infusions on disease activity related serum cytokine, growth factor and soluble adhesion molecule levels in patients with SSc-associated Raynaud's phenomenon.

MATERIAL AND METHODS

Patient selection and clinical evaluation

Serum samples were obtained from 12 patients with SSc before and after 5–7 days of intravenous iloprost infusions at a dosage of 0.5–2.0 ng/kg/min over 6–8 h/day. All patients fulfilled the criteria proposed by the American College of Rheumatology and did not have features diagnostic of other connective tissue diseases (7). There were 5 women and 7 men, with a mean \pm SD age of 49.8 \pm 12.1 years (range 32–75). The patients were grouped according to the classification system proposed by LeRoy et al. (7): 4 patients had limited cutaneous SSc (lSSc) and 8 had diffuse cutaneous SSc (dSSc). Patients details are given in Table I.

Measurement of circulating adhesion molecules

Circulating sICAM-1, sVCAM-1, sE-selectin, VEGF and endothelin-1 (ET-1) were measured following a quantitative sandwich enzyme immunoassay technique using commercial immunoassay kits (R&D, Oxford) in accordance with the manufacturer's instructions. The results shown are means for replicate wells; standard curve and positive control samples were included in each assay run.

Control samples from healthy adults using these assay kits were found to have the following endogenous levels given as 95% confidence intervals and considered as normal values: sICAM-1 – 115–306 ng/ml (mean value: 211); sVCAM-1 – 395–714 ng/ml (mean value: 553); sE-selectin – 29.1–63.4 ng/ml (mean value: 46.3); VEGF – 62–707 pg/ml (mean value: 220) and human ET-1 – 0.71–2.3 pg/ml (mean value: 1.2).

According to the manufacturer's instructions, the minimum detectable dose of sICAM-1 is less than 0.35 ng/ml of sVCAM-1 2.0 ng/ml, of sE-selectin 0.1 ng/ml; of VEGF 9.0 pg/ml and of human ET-1 0.16 pg/ml.

Table I. Clinical and laboratory data of patients with systemic sclerosis (SSc) before and after iloprost therapy

Patient	Age/sex	Subset	Lung fibrosis	Oesophageal dysfunction	Anti-nuclear antibody	Anti-topoisomerase I antibody	Anti-centromere antibody	sICAM-1 (ng/ml)		sVCAM-1 (ng/ml)		VEGF (pg/ml)		sE-Selectin (ng/ml)		ET-1 (pg/ml)	
								Before	Post	Before	Post	Before	Post	Before	Post	Before	Post
1	37/w	dSSc	+	+	+	-	-	752	625	752	640	100	80	131	87	2.01	2.25
2	40/w	dSSc	+	+	+	-	-	995	927	881	763	207	283	87	85	1.53	1.71
3	42/m	dSSc	+	+	+	+	-	386	355	999	442	193	168	44	37	1.75	1.26
4	45/m	dSSc	+	+	+	+	-	713	645	744	986	441	405	180	167	2.53	2.05
5	50/m	dSSc	+	+	+	+	-	227	192	486	467	293	262	66	43	1.67	1.56
6	50/m	dSSc	-	+	+	+	-	766	662	864	722	62	69	182	164	3.92	3.32
7	58/w	dSSc	+	+	+	-	-	441	324	921	909	95	70	53	34	2.49	1.46
8	64/m	dSSc	+	-	+	-	-	785	692	968	906	167	109	90	78	1.43	0.51
9	32/w	ISSc	-	-	+	+	+	408	298	1129	816	84	66	192	155	0.97	1.18
10	43/m	ISSc	-	-	+	+	-	617	538	874	636	327	219	151	113	3.21	2.20
11	62/w	ISSc	-	+	+	-	+	348	298	1003	710	213	216	118	62	0.64	0.65
12	75/m	ISSc	+	+	+	+	-	439	397	893	885	23	26	82	66	1.62	1.38

ISSc: limited cutaneous SSc; dSSc: diffuse cutaneous SSc.

Statistical analysis

Statistical analysis was performed using the Wilcoxon rank sum test. P-values < 0.05 were considered to be statistically significant.

RESULTS

The therapy of repeated iloprost infusions was well tolerated by all patients. The most common side effects appeared to be flushing, nausea and headache. Most side effects were readily manageable by dose reduction. All patients reported on a marked reduction of number and severity of Raynaud's attacks during infusions and during the following 6-8 weeks.

Pre- and post-iloprost therapy levels of sICAM-1, sVCAM-1, sE-selectin, VEGF and human ET-1 obtained in the various samples are given in Table I, demonstrating that there was considerable variation of these levels in each patient. Serum levels of sICAM-1, sVCAM-1, sE-selectin and partially VEGF and ET-1 were prominently elevated in patients with ISSc as well as dSSc. There was no significant difference among these subsets.

The basal expression of sICAM-1 was markedly upregulated at both time-points - before and after iloprost therapy. It was significantly lower after iloprost therapy ($p < 0.0001$; Fig. 1a). Serum levels of sVCAM-1 were also significantly reduced after iloprost therapy ($p < 0.02$; Fig. 1b). Furthermore, the patients exhibited significantly reduced levels of sE-selectin after iloprost therapy compared to measurements before treatment ($p < 0.0001$; Fig. 1c).

The serum concentrations of VEGF and vasoactive peptide human ET-1 revealed decreased levels after therapy too (Fig. 1d and e): 8 of 12 patients presented a lower VEGF concentration and a decreased human ET-1 concentration after iloprost therapy.

Measurements for internal organ involvement and autoantibodies are also listed in Table I. There was no association between these parameters and *in vivo* expression of proinflammatory adhesion molecules.

DISCUSSION

Raynaud's phenomenon is a frequent and early component of SSc. It represents the clinical correlate of a disturbed endothelial cell function. The endothelial cells are activated by autoantibodies, T-cell granzymes and other mechanisms that are not fully understood. This activation leads to an increased expression of adhesion molecules at the surface of the endothelial cells, which will subsequently release these molecules as soluble factors into the serum. Thus, soluble E-selectin, sVCAM-1 and sICAM-1 are generated by the endothelial cells under the influence of proinflammatory enzymes and released into the serum (8). E-selectin is only expressed by and released from previously activated endothelial cells. Soluble VCAM-1 is mainly released from endothelial cells; however, it can also derive from epithelial cells, dendritic cells and macrophages. On the other hand, soluble ICAM-1 can be synthesized not only from endothelial cells, but also from epithelial cells, granulocytes, hepatocytes and smooth muscle cells (9). In skin lesions from SSc, VCAM-1 has been demonstrated in endothelial cells, while ICAM-1 was found in fibroblasts too. Interestingly, serum levels correlated with *in situ* expression and the clinical activity of the disease (3).

The stable prostacyclin-analogue iloprost is well

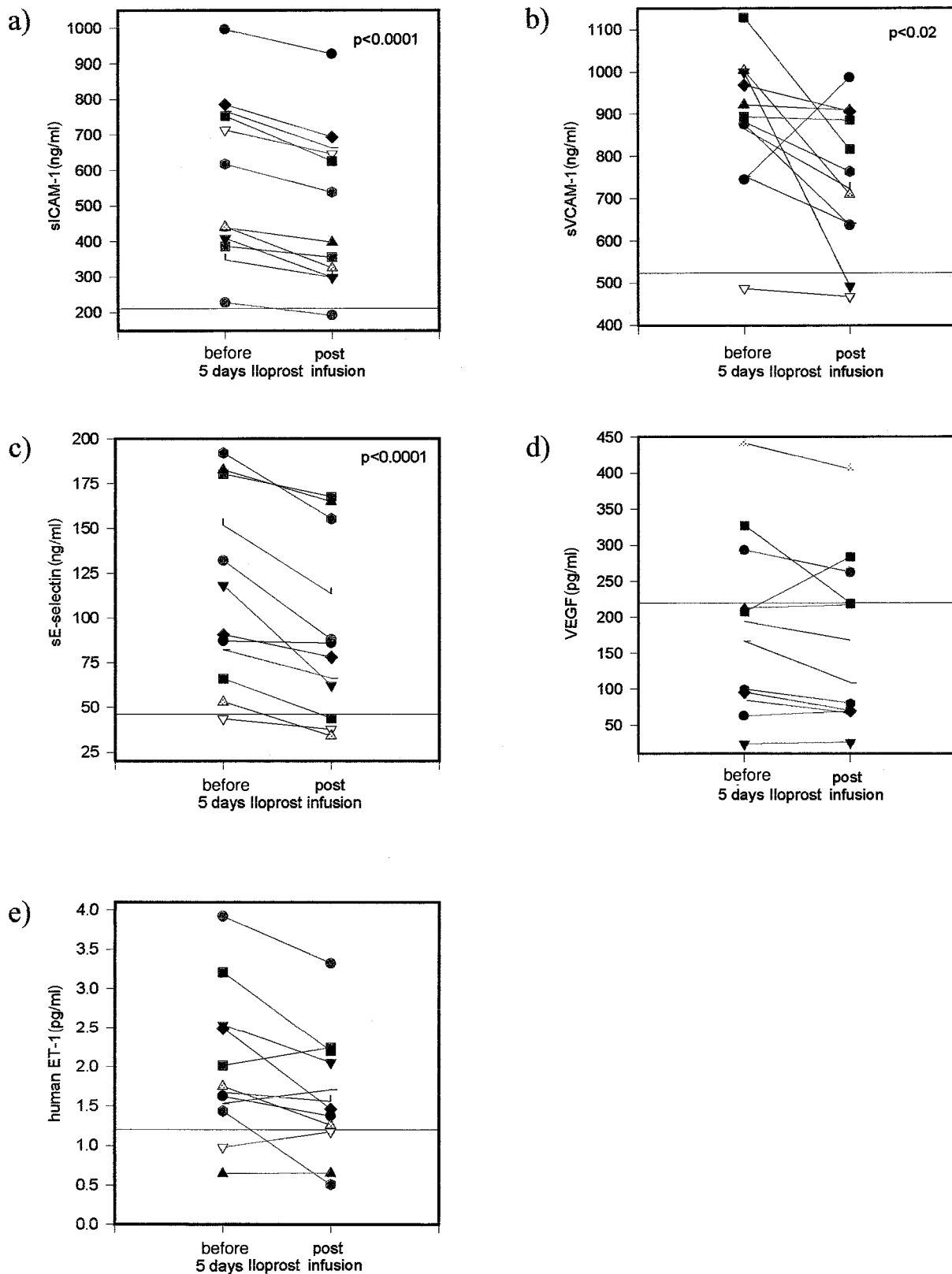


Fig. 1a-e. Iloprost therapy can significantly reduce the serum levels of sICAM-1 (a), sVCAM-1 (b) and sE-selectin (c), whereas the serum levels of VEGF (d) and endothelin 1 (ET-1) (e) are changed but to a less extent. All patients were examined before and 5-7 days after therapy. The serum levels were determined in triplicate and were indicated as mean values. The line represents the cut-off value from normal donors (mean + 1 SD) according to the manufacturer's declaration.

established for the therapy of Raynaud's phenomenon in SSc. The complex pharmacological effects consist of numerous changes: (a) direct vasodilatory activity affecting arteries and veins, (b) inhibition of the ADP-dependent platelet aggregation, (c) reduction in the release of vasoconstrictory mediators, (d) anti-inflammatory effects due to a reduction of TNF α -synthesis, (e) subsequently reduced adherence of neutrophil granulocytes at the endothelial cells of the vessel wall. The clinical effect of iloprost on Raynaud's phenomenon is remarkable, and usually persists for several weeks, a duration which is difficult to explain at present. In accordance with other investigations (1–3), our patients exhibited increased serum concentrations of soluble adhesion molecules ICAM-1, VCAM-1 and E-selectin before therapy which have been significantly reduced by treatment with iloprost.

These changes reflect the reduced activation of endothelial cells and might help to explain the clinical effects. Della Bella et al. (10) have shown that iloprost, in contrast to nifedipine, can inhibit the IL-1 β -production of circulating mononuclear cells. Similar to the reduction of TNF α -synthesis by iloprost, these findings might explain the effects observed in the immune cells.

ET-1 is one of the most potent vasoconstrictors. In SSc patients it was found in increased concentrations in serum as well as in bronchoalveolar lavage fluids. In addition, it modulates fibroblast function and can upregulate the expression of ICAM-1 in the cells (11). Increased concentrations of ET-1 have only been observed in four patients, while two-thirds of the patients experienced a reduction after the therapy. The downregulation in even non-pathologically increased values might modulate the balance between vasoconstrictory and vasodilatory factors in favour of the latter in Raynaud's phenomenon.

The VEGF is very important during angiogenesis, which plays a significant role in the development of malignant tumours and in wound healing. While in rheumatoid arthritis increased serum concentrations were detected, in SSc, and particularly in our patients, concentrations were within the normal range. This phenomenon remains unexplained, since blood circulation disturbances in SSc as well as the increased concentrations of TGF α are potent inducers of VEGF (12).

As a whole, our study has shown that infusion therapy with the stable prostacyclin analogue iloprost leads to an improvement of Raynaud's phenomenon in association with a normalization of increased endothelial cell activity. More specifically, we observed a significant decrease in concentrations of the soluble adhesion molecules ICAM-1, VCAM-1 and E-selectin,

which correlates well with the *in situ* expression of these molecules in endothelial cells, as shown in former studies (3). Therefore, in addition to its vasoactive effects, iloprost has the potential to modulate inflammatory processes in SSc via inhibition of adhesion molecule mediated endothelial cell–leukocyte interactions.

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