Treatment of Raynaud's Phenomenon with Intravenous Prostaglandin E_1α-Cyclodextrin Improves Endothelial Cell Injury in Systemic Sclerosis

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ABSTRACT. ***Objective***. To evaluate the efficacy and safety of prostaglandin (PG) E_1α-cyclodextrin for Raynaud's phenomenon (RP) secondary to systemic sclerosis (SSc) and its effect on variables of immune activation and endothelial injury in SSc such as tumor necrosis factor-α (TNF-α), soluble interleukin 2 receptor (sIL-2R), circulating intercellular adhesion molecule-1 (cICAM-1), von Willebrand factor (vWF), and tissue-type plasminogen activator (t-PA).

Methods. We studied 36 women with SSc, 24 of them given three 60 μg intravenous PGE_1α-cyclodextrin infusions on 5 consecutive days at 6 week intervals during the winter. RP symptoms and healing of digital lesions were evaluated. Twenty age matched healthy women were the controls. TNF-α, sIL-2R, cICAM-1, vWF, and t-PA were measured after the first and last infusion of PGE_1α-cyclodextrin and correlated with clinical features.

Results. RP symptoms improved in 87% of the patients. The benefit of each 5 day cycle lasted 4 or more weeks in 75%. PGE_1α-cyclodextrin reduced the daily frequency of RP symptoms by 20% ($p < 0.05$), 41% ($p < 0.005$), and 53% ($p < 0.0005$) from baseline after the 1st, 2nd, and 3rd infusions, respectively. The severity of the attacks was reduced to a limited degree. In 12 of the 14 patients with digital lesions, these healed completely. Ten patients had mild side effects during treatment (headache, increased intestinal motility, flushing). TNF-α, sIL-2R, cICAM-1, vWF, and t-PA plasma concentrations were significantly higher in patients with SSc than controls ($p < 0.05$, $p < 0.001$). TNF-α, sIL-2R, and cICAM-1 were higher in diffuse SSc and patients with lung involvement. The plasma levels of cICAM-1 and t-PA were significantly reduced after the 1st infusion of PGE_1α-cyclodextrin (both $p < 0.005$) and further reduced after the last ($p < 0.0005$ and $p < 0.005$).

Conclusion. PGE_1α-cyclodextrin reduces RP symptoms and plasma levels of the markers of endothelial injury in SSc, suggesting that an improvement of endothelial dysfunction contributes to its prolonged therapeutic effect. (J Rheumatol 2001;28:786-94)

Key Indexing Terms:
RAYNAUD'S PHENOMENON SYSTEMIC SCLEROSIS PROSTAGLANDINS
INTERCELLULAR ADHESION MOLECULE-1 TISSUE-TYPE PLASMINOGEN ACTIVATOR

More than 90% of patients with systemic sclerosis (SSc) have Raynaud's phenomenon (RP)[1]. The digital arteries of patients with scleroderma are restricted because of hyperplasia and fibrosis of the intima[2]. When patients are exposed to cold or stress, vasoconstriction is superimposed on the narrowing and causes occlusion of the lumen and tissue ischemia. Patients with SSc present an enhanced sympathetic response to cold[3] and the release of vasoconstricting mediators from activated platelets[4] during the attacks of RP.

Since prostanoids dilate arterioles, capillaries and postcapillary venules, inhibit platelet aggregation, and may have fibrinolytic activity, they have been used to treat RP[5]. Natural derivatives of arachidonic acid [prostaglandin I_2 (PGI_2) or prostacyclin] and dihomo-γ-linolenic acid (PGE_1 or alprostadil) reduced the frequency and severity of vasospasm in many open and double-blind crossover trials[6-9]. These compounds, however, cause irritation at the site of injection and, being chemically unstable at 37°C and neutral pH, have a very short half-life[5,6]. PGE_1, moreover, undergoes substantial inactivation while passing through the pulmonary circulation[10]. To overcome these drawbacks, which limit the therapeutic potential of prostanoids, chemically stable analogs of PGI_2 and PGE_1 have been synthesized. They retain the vasodilating properties and inhibit *in vitro* platelet aggregation more efficiently than the natural compounds on a molar basis[11,12]. They can be injected into a peripheral vein so that

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patients with RP can be periodically treated in outpatient departments. This treatment is based on the fact that the benefit of natural and synthetic prostaglandins persists up to 4 to 6 weeks. Iloprost, the carbacyclin derivative of PGI_2, has been the most extensively studied so far. Several clinical studies have demonstrated its efficacy on primary and secondary RP in a large number of patients[13-18].

Stable analogs of PGE_1 have also been synthesized. PGE_1 has been inserted in liposomes or conjugated with carbohydrates (dextrins) to render the molecule more soluble and protect it from inactivation in the pulmonary circulation[19,20]. Two preliminary reports indicated that PGE_1α-cyclodextrin has potential therapeutic benefit on RP[21,22].

We report our encouraging clinical experience with the infusion of PGE_1α-cyclodextrin in a group of 24 women with SSc and severe RP. A dose of 60 μg of PGE_1α-cyclodextrin, on 5 consecutive days, repeated 3 times at 6 week intervals, significantly reduced the frequency and duration of RP attacks during the winter in more than 80% of the patients.

We also investigated whether the lasting therapeutic effect of PGE_1α-cyclodextrin was related to a functional improvement of endothelial cells by measuring plasma levels of markers of endothelial damage such as the soluble circulating form of intercellular adhesion molecule-1 (cICAM-1), tissue-type plasminogen activator (t-PA), and von Willebrand factor (vWF). Plasma concentrations of cICAM-1, t-PA, and vWF are reportedly high in SSc[23-26]. Tumor necrosis factor-α (TNF-α) and soluble interleukin 2 receptor (sIL-2R) were also measured, as the former induces the expression of ICAM-1[27] on endothelial cells and the latter is considered indicative of lymphocyte activation in SSc[28].

MATERIALS AND METHODS

Patients and controls. Thirty-six women (median age 62 yrs, range 33–74) with the clinical and serological features of SSc were studied. All fulfilled the American Rheumatism Association criteria for SSc[29] and gave their informed consent to the study. Twenty-seven had sclerodactyly or involvement of the skin of hands, forearms, and face ("limited scleroderma") and 9 had widespread thickening of the skin ("diffuse scleroderma"). Twenty age matched healthy women (median age 56 yrs, range 40–61) were studied as controls. Table 1 summarizes the clinical and laboratory variables of the case list. The involvement of internal organs was evaluated as follows. Lung: DLCO ≤ 70% or FEV_1, FVC, TLC ≤ 75% of predicted, or presence of interstitial fibrosis at chest radiography; esophagus: signs of esophagitis at endoscopy or hypotonia of lower esophageal sphincter and altered peristalsis at manometry, or evidence of gastric reflux at pHmetry; heart: arrhythmias requiring medication, or presence of pericardial effusion, alteration of parietal kinesis at 2 dimensional echocardiogram; kidney: abnormalities of serum urea nitrogen (> 40 mg/dl) and/or creatinine (> 1.5 mg/dl). Skin involvement was evaluated according to Rodnan's modified score[30].

Nailfold capillaroscopy (using a Zeiss STEMI SV 6 stereomicroscope, magnification 20× to 125×) was done in all patients; 20 showed a "slow," 10 an "active" pattern according to Maricq[31]. Six had a "nonspecific" pattern.

Twenty-four were treated with PGE_1α-cyclodextrin in the hospital outpatient department because of recurrent severe vasospasm; 12 were not treated because 5 had mild RP that could be managed with the usual precautions, 2 had myocardial ischemia, and 5 refused treatment. Sixty μg of PGE_1α-cyclodextrin (Prostavasin®, Schwartz-Pharma, Milan, Italy) were diluted in 250 ml of 0.9% NaCl solution and infused at 20 μg/h (i.e., about 4–6 ng/kg/min, according to patient body weight) into a peripheral vein. The infusion was repeated on 5 consecutive days, 3 times at 6 week intervals during the winter months of 1998–99 (first cycle between mid-November and mid-December, second between the beginning of January and mid-February, third between the end of February and the end of March).

Table 1. Main clinical and laboratory features of patients with systemic sclerosis.

	All SSc n = 36	Limited SSc n = 27	Diffuse SSc n = 9	p*
Years from Raynaud onset	11.5 (2–51)	11.0 (2–51)	14 (4–43)	NS
Years from SSc diagnosis	4.0 (1–26)	3.0 (1–26)	6.0 (4–17)	< 0.05†
Skin score (Rodnan)	13 (0–33)	11 (0–26)	25 (9–33)	< 0.005†
Digital lesions	14/36	9/27	6/9	NS
Internal organ involvement				
Esophagus	32/36	23/27	9/9	NS
Lung	10/36	2/27	8/9	< 0.005*
Heart	3/36	2/27	1/9	NS
Kidney	0/36	0/27	0/9	NS
Anticentromere Ab	26/36	20/27	6/9	NS
Scl-70 Ab	4/36	1/27	3/9	NS
Ro/SSA Ab	5/36	5/27	0/9	NS
La/SSB Ab	1/36	1/27	0/9	NS

Limited vs diffuse SSc (†Mann-Whitney U test; * chi-square test).

The daily temperatures provided by the weather report during these months were recorded to disentangle the effect of treatment from that of the climate. The mean (± standard deviation) daily temperatures for the fortnights before and after each cycle of PGE_1α-cyclodextrin infusion are reported in Table 2. Four patients (LT, AM, GA, GM) skipped one treatment cycle because of viral upper respiratory infections. Two of them (GA, GM) were treated twice at an 8 week interval.

Patients kept diaries indicating the daily frequencies of vasospasm. Each attack was arbitrarily graded (from 0 to 10) using visual analog scales. Patients were taught to rate attacks according to the duration and severity of the vasospasm and the functional impairment they experienced. This information was provided for the fortnights before and after every cycle of therapy. The mean daily number of attacks and the mean severity of the attacks were calculated for each 2 week period.

All patients completed questionnaires after each 5 day cycle to assess the therapeutic efficacy of PGE_1α-cyclodextrin. They had to indicate whether they perceived an overall improvement of their clinical condition and, specifically, if they had fewer or shorter attacks and whether they experienced less pain during vasospasm. If they benefited they were asked to estimate how long (in weeks) the effect lasted after each 5 day infusion. Lastly, they compared the efficacy of PGE_1α-cyclodextrin with other drugs they had previously used to treat RP.

Laboratory analysis. Blood samples for laboratory analysis (including TNF-α, cICAM-1, sIL-2R, vWF, t-PA) were collected before and after first and last cycles of PGE_1α-cyclodextrin. Controls and patients with SSc who were not treated with PGE_1α-cyclodextrin gave a sample once, at the beginning of the study.

Blood samples were collected in tubes containing disodium ethylenediaminotetraacetic acid (Na_2 EDTA, 0.2 M) for TNF-α and cICAM-1 or trisodium citrate (0.13 M) for sIL-2R, t-PA, and vWF. Samples were centrifuged at 3000 rpm for 15 min at room temperature (22°C). Plasma was separated and stored in aliquots at –80°C until assayed.

Table 2. Mean (± SD) temperatures (˚C) registered during the fortnights before and after each cycle of PGE_1 α-cyclodextrin.

Patients	No.	1st Cycle		2nd Cycle		3rd Cycle	
		Before	After	Before	After	Before	After
CG, SM, MR	3	10 ± 2˚	4 ± 1˚	3 ± 2˚	4 ± 2˚	3 ± 3˚	8 ± 2˚
LT	1	10 ± 2˚	4 ± 1˚	3 ± 2ˆ	4 ± 2˚	—	—
BC	1	7 ± 3˚	4 ± 2˚	5 ± 1˚	4 ± 4˚	9 ± 2˚	11 ± 2˚
MA, CR, DM	3	7 ± 3˚	4 ± 2˚	5 ± 1˚	4 ± 2˚	6 ± 3˚	10 ± 2˚
PC, VR, BR, LS, QG	5	5 ± 2˚	4 ± 3˚	5 ± 2˚	4 ± 3˚	8 ± 1˚	10 ± 2˚
MA	1	5 ± 2˚	4 ± 3˚	4 ± 2˚	4 ± 4˚	10 ± 2˚	12 ± 2˚
BG, FR, FP	3	4 ± 2˚	3 ± 1˚	4 ± 2˚	4 ± 4˚	10 ± 2˚	12 ± 2˚
QE, CF	2	5 ± 2˚	3 ± 2˚	5 ± 4˚	5 ± 3˚	9 ± 2˚	11 ± 2˚
GA	1	4 ± 2˚	4 ± 2˚	—	—	8 ± 1˚	10 ± 2˚
MC	1	4 ± 2˚	3 ± 2˚	4 ± 2˚	4 ± 4˚	10 ± 2˚	12 ± 2˚
GM	1	4 ± 2˚	3 ± 1˚	—	—	8 ± 1˚	10 ± 2˚
AM	1	5 ± 2˚	4 ± 2˚	5 ± 2˚	4 ± 3˚	—	—
MM	1	5 ± 2˚	4 ± 3˚	5 ± 2˚	4 ± 3˚	9 ± 2˚	11 ± 2˚

Commercial ELISA methods were used to measure TNF-α, sIL-2R, and cICAM-1 (Medgenix EASIA™, Biosource Europe, Nivelles, Belgium) and t-PA antigen (American Diagnostica, New York, NY, USA) according to manufacturer's instructions. vWF was measured in plasma by ELISA according to the method used in the laboratory of the "Centro Emofilia e Trombosi Bianchi Bonomi" of Milan University which employs the Mab 7D10.E10 to catch vWF and Mab 11B6.18 conjugated with horseradish peroxidase for detection. A 96 well microplate Gralis Reader spectrophotometer (SLT Labinstruments GmbH, Salzburg, Austria) was used for the assays.

The interassay coefficients of variation (CV) were 12% for TNF-α, 5% for sIL-2R, 6% for cICAM-1, 10% for vWF, and 10% for t-PA. All the assays were run in duplicate. The CV of duplicate determinations was less than 8%.

Statistical analysis. Statistical analysis was done using nonparametric tests because of the non-Gaussian distribution of the variables. Differences between the prevalence of internal organ involvement or digital lesions in limited and diffuse scleroderma were analyzed by the chi-squared test. The significance of the differences between laboratory variables of patients with SSc and controls, or pairs of subsets of disease were analyzed by Mann-Whitney U test. The effect of treatment was evaluated by comparing clinical and laboratory variables over time by Friedman's nonparametric 2 way analysis of variance. Paired comparisons over time (between pretreatment and posttreatment data) were done thereafter with the Wilcoxon matched-pairs signed-rank test. A p value < 0.05 was considered significant.

RESULTS

Clinical effect of PGE_1α-cyclodextrin treatment. The 3 cycles of infusion with PGE_1α-cyclodextrin were judged overall to have been an effective treatment for RP by 87%, 86%, and 95% of the patients. Table 3 summarizes patients' subjective assessments of the effect on the frequency and duration of digit vasospasm and on pain during the attacks.

The beneficial effect of the first 5 day infusion lasted 4 weeks or more in 18 of 24 patients (75%). In 13 the effect was still present 6 weeks later, when the 2nd cycle of infusion was given. The percentages of patients who had a long-lasting therapeutic effect (4 weeks or more) were 72% and 76% after the 2nd and the 3rd cycles.

Nineteen of the 24 patients had already received one or more drugs for the RP. Nine had taken oral or transdermal nitrates (isosorbide dinitrate 10 mg tid or isosorbide-5-mononitrate SR 60 mg once daily, transdermal nitroglycerine 5 mg/daily); 8 had taken calcium channel blocking agents (nifedipine XL 30 mg once daily or diltiazem 60 mg bid), 9 ketanserine (20 mg bid). Eighteen of these 19 considered PGE_1α-cyclodextrin more effective than previous treatments to control vasospasm.

Table 3. Number of patients (%) who reported clinical improvement after infusion of PGE_1 α-cyclodextrin.

	1st Cycle	2nd Cycle	3rd Cycle
Overall	21/24 (87)	19/22 (86)	21/22 (95)
Frequency of attacks	17/24 (71)	18/22 (82)	20/22 (91)
Length of attacks	20/24 (83)	21/22 (95)	22/22 (100)
Pain during attack	12/24 (50)	16/22 (73)	14/22 (63)

Only 17 of the 24 patients completed their diaries to provide an objective assessment of the effect of PGE_1α-cyclodextrin on the frequency and severity of RP attacks. Figure 1 shows the mean (± SD) daily frequency of RP and the mean (± SD) attack severity score in the six 2-week observation periods (i.e., the fortnights before and after each of the three 5-day infusions). The number of attacks per day was significantly reduced after the first cycle of PGE_1α-cyclodextrin and decreased further after the second and the third 5-day infusions. The frequency of the attacks was reduced, respectively, by 20%, 41%, and 53% compared to baseline.

The mean severity of the attacks in every 2 week period was only marginally, although significantly, affected, being reduced by 17% after the second cycle and by 21% after the third. The higher mean daily temperature in the fortnights before and after the third cycle (Table 2) may have contributed

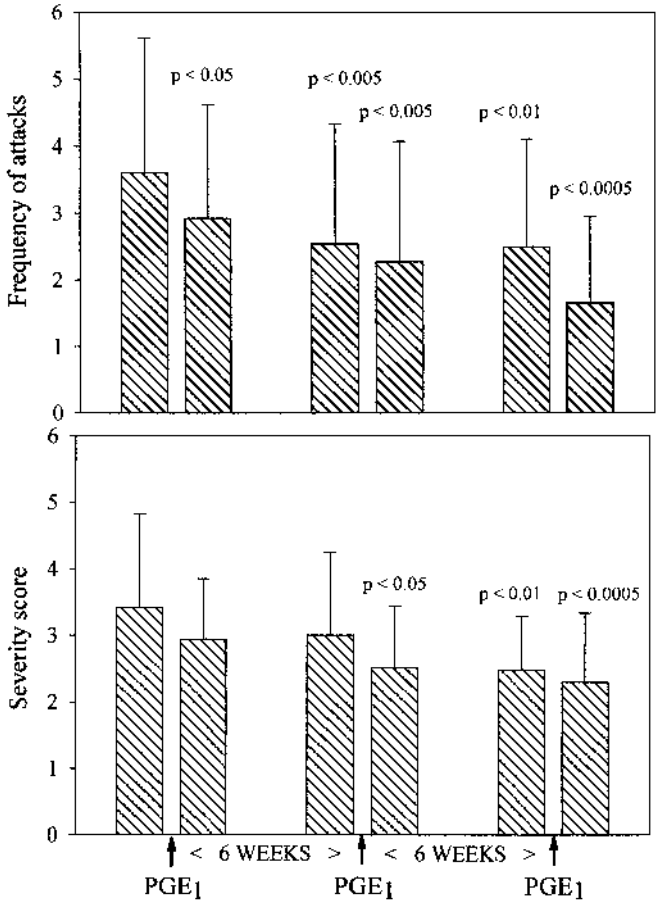

Figure 1. Mean (± SD) daily frequency and severity score of Raynaud attacks in the fortnights before and after each infusion of PGE_1α-cyclodextrin. The significance of differences between means for each 2 week period and the fortnight before the first infusion (baseline) is indicated (Wilcoxon matched signed-rank test).

to reducing the frequency and severity of RP attacks in those periods.

Twelve of the 14 patients who had acral ulcers at the beginning of the study showed complete healing of the digital lesions.

Side effects consisted in headache (4 patients), increased intestinal motility (4 patients), and flushing (2 patients). These symptoms were limited to the period of infusion and did not require drug suspension. In 6 patients who had a reaction at the injection site, the infusion rate had to be slowed from 20 μg to 10 μg/h. No significant hypotension (> 20 mm Hg drop of systolic or diastolic blood pressure) was observed during the infusions.

Immune activation and endothelial damage in SSc: correlation with clinical subsets of disease. TNF-α, sIL-2R, cICAM-1, vWF, and t-PA were measured in 36 SSc patients with RP and in 20 controls. SSc patients had significantly higher levels of TNF-α, sIL-2R, cICAM-1, vWF, and t-PA than age matched healthy women (Figure 2). Table 4 shows the mean levels of TNF-α, sIL-2R, cICAM-1, vWF, and t-PA in different subsets of SSc patients. We found no significant correlation between any of these variables and the duration of disease (i.e., number of years from onset of RP or from a definite diagnosis of SSc) or any significant differences between patients with "early onset" scleroderma (< 2 yrs) and those with long-lasting disease. Patients with diffuse scleroderma tended to have higher levels of TNF-α, sIL-2R, and cICAM-1 than those with limited disease and these same variables were significantly higher in those with lung complications than in those without ($p < 0.05$).

Effect of PGE_1α-cyclodextrin treatment on immunologic variables. Twenty-four of 36 patients were treated with PGE_1α-

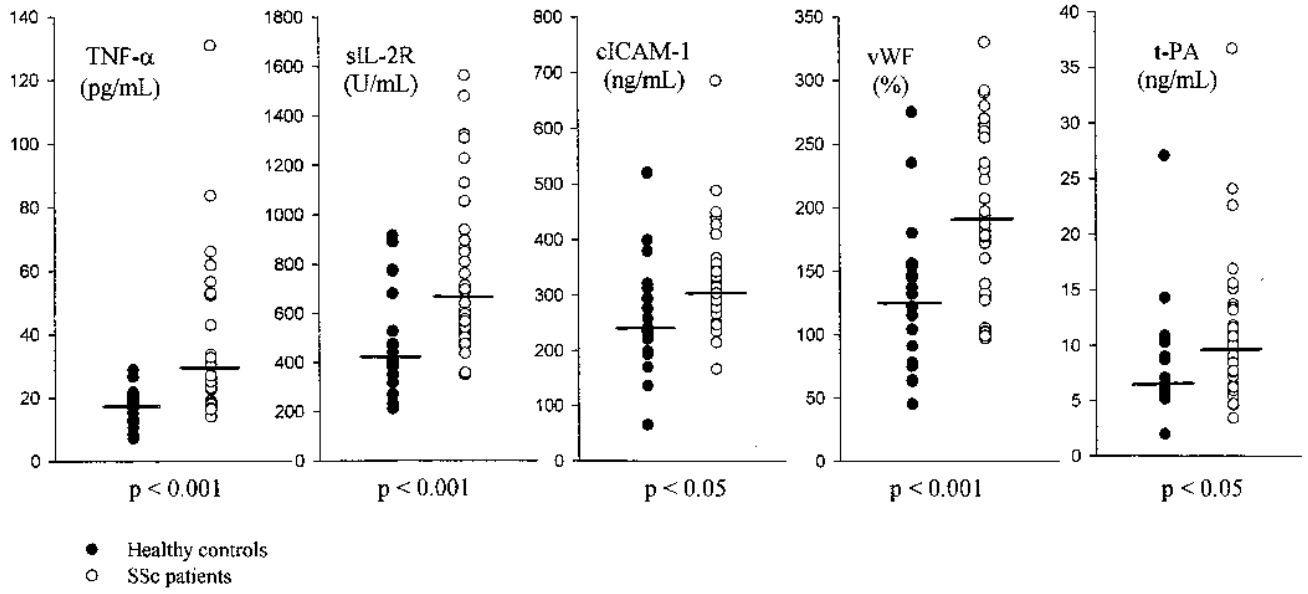

Figure 2. Plasma levels of TNF-α, sIL-2R, cICAM-1, vWF, and t-PA in 36 patients with SSc and 20 healthy age matched women. The significance of the differences between the 2 groups is indicated (Mann-Whitney U test).

Table 4. Plasma levels of TNF-α, sIL-2R, cICAM-1, vWF, and t-PA in different subsets of patients with systemic sclerosis. Data are median (range).

	No.	TNF-α pg/ml	sIL-2R, U/ml	cICAM-1, ng/ml	vWF, %	t-PA ng/ml
All	36	25 (14–131)	629 (352–1566)	299 (166–685)	187 (97–330)	9.1 (3.4–36.7)
Early (< 2 yrs)	9	26 (16–53)	598 (506–1482)	290 (240–349)	182 (127–265)	7.2 (4.7–11.6)
Late	27	25 (14–131)	643 (352–1566)	303 (166–685)	194 (97–330)	9.5 (3.4–36.7)
Limited	27	25 (14–66)	598 (352–1566)	290 (166–685)	187 (97–292)	9.1 (4.6–36.7)
Diffuse	9	30 (18–131)	811 (469–1326)	342 (214–449)	175 (98–330)	7.7 (3.4–24.1)
Internal organ involvement[†]						
Esophagus						
Yes	32	26 (14–131)	629 (352–1566)	294 (166–685)	187 (97–330)	8.4 (3.4–36.7)
No	4	26 (17–33)	598 (439–1482)	302 (246–314)	223 (178–280)	10.3 (9.0–13.4)
Lung						
Yes	10	53* (18–131)	971° (469–1566)	362¶ (214–685)	157 (98–330)	8.1 (3.4–24.1)
No	26	25* (14–57)	583° (352–1482)	290¶ (166–488)	190 (97–292)	9.1 (4.6–36.7)
Heart						
Yes	3	18 (17–66)	643 (439–1566)	303 (214–685)	280 (102–290)	10.4 (6.2–13.4)
No	33	26 (14–131)	615 (352–1482)	298 (166–488)	187 (97–330)	8.8 (3.4–36.7)

[†]No patient had renal scleroderma.
[#°*]p < 0.05 (Mann-Whitney U test).

cyclodextrin. They were representative of the whole SSc group as "limited" and "diffuse" forms of the disease were represented similarly to the whole group (19 and 5 cases). The duration of disease (13.6 ± 10.5 yrs from the onset of RP and 5.9 ± 6.0 yrs from SSc diagnosis), the mean skin score (15.4 ± 9.0), the presence of digital lesions (13/24), and the number of patients with internal organ involvement (esophagus 22/24, lung 6/24, heart 1/24) were also similar.

The baseline laboratory findings and those after the first and the last cycles of infusion are reported in Table 5. Baseline values of the SSc patients treated with PGE_1α-cyclodextrin were not significantly different from those of all SSc patients. The first infusion significantly reduced cICAM-1 and t-PA, and their concentrations decreased further after the third infusion. Plasma levels of cICAM-1 and t-PA after the last treatment were similar to those in healthy controls. A significant reduction of sIL-2R levels was observed in the plasma collected after the last treatment. The individual values of cICAM-1 and t-PA before and after the first and last cycles are reported in Figure 3.

Table 5. Plasma levels of TNFα, sIL-2R, cICAM-1, vWF, t-PA before and after 1st and last cycles of PGE_1 α-cyclodextrin. Data are median (range).

	Baseline	After 1st Cycle	After 3rd Cycle
TNFα, pg/ml	25 (14–84)	25 (12–63)	22 (13–50)
sIL-2R, U/ml	589 (352–1566)	578 (338–1874)	529* (272–1596)
cICAM-1, ng/ml	306 (234–685)	268** (197–509)	227*** (179–466)
vWF, %	185 (97–290)	175 (86–280)	156 (87–325)
t-PA, ng/ml	8.0 (3.4–36.7)	6.9** (3.4–15.0)	6.3** (1.0–16.3)

*p < 0.05; **p < 0.005; ***p < 0.0005 vs baseline (Wilcoxon matched pairs signed-rank test).

DISCUSSION

Our study provides 2 main conclusions: (1) infusion of PGE_1α-cyclodextrin causes sustained clinical benefit in patients with RP secondary to SSc with limited side effects; (2) the reduced frequency of vasospasm coincides with lower plasma concentrations of cICAM-1 and t-PA, 2 markers indicative of endothelial damage in SSc.

The favorable effects of PGE_1α-cyclodextrin are clear from the significant reduction of the number of vasospasms per day after infusion of the drug, by the healing of digital

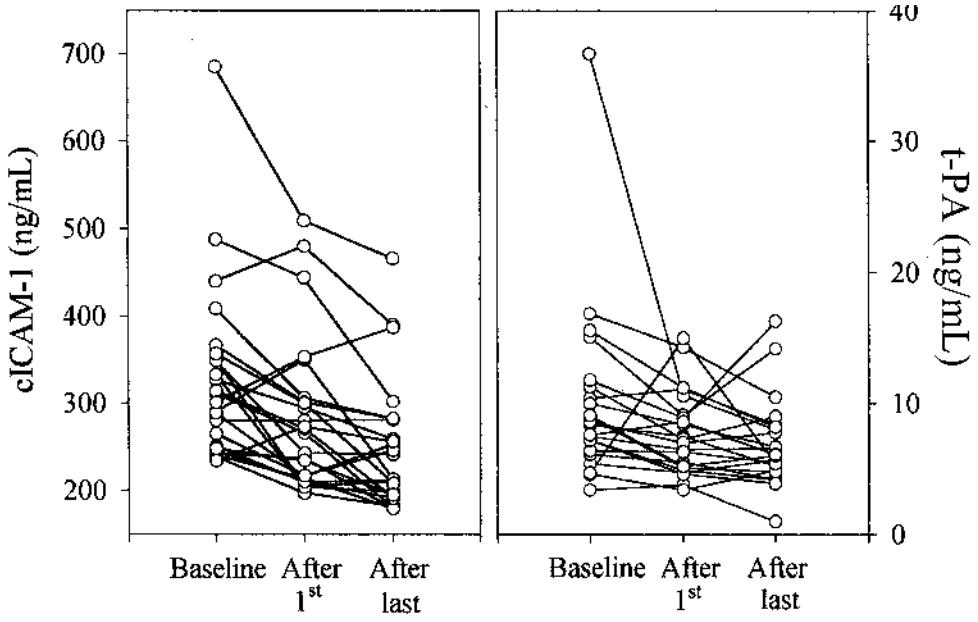

Figure 3. Individual plasma levels of cICAM-1 and t-PA in 24 patients with SSc treated with $PGE_1\alpha$-cyclodextrin. The levels of cICAM-1 and t-PA measured in plasma collected before and after the 1st and last 5 day infusion are illustrated.

ulcers in 85% of patients, and, to a lesser extent, by a reduction in the severity of symptoms during the attacks (see Figure 1 and Table 3). Most patients reported sustained subjective improvement of symptoms and there was no evidence of tachyphylaxis after 3 treatment cycles.

A reduction in the frequency of vasospasm may occur spontaneously in untreated patients as the temperature rises from winter to summer[3la]. In our trial, the climate did not appear to play any role in the clinical improvement after the first 2 cycles of infusions, as the mean temperatures in the 2 weeks after the infusions were the same as or lower than in the fortnights before the study. The rise in temperature may, however, have contributed to the further reduction of the number and severity of the attacks after the last $PGE_1\alpha$-cyclodextrin infusion.

The frequency of RP attacks dropped 41% from baseline after the second infusion during the winter. Similar results have been reported with oral calcium antagonists (drops of 27 to 48% with nifedipine, 33 to 64% with diltiazem) and ketanserin (34 to 60%)[32-37]. Mohrland, *et al*[9] reported a 56% reduction of attacks with a 3 day continuous infusion of 10 ng/kg/min of PGE_1 and Belch, *et al*[7] noted a 75% drop in attacks with intermittent infusion of 7.5 ng/kg/min of PGI_2 for 5 h once a week for 3 weeks. However, since native prostaglandins have been virtually abandoned for the treatment of RP because of their therapeutic limits, it appears more interesting to compare our results with the several studies employing iloprost, which is the standard treatment for severe RP. Iloprost reduced the frequency of attacks by 25–55%[13-18]. However, rather more patients had side effects with iloprost (78 to 100% in the 4 studies with the largest case lists) than with $PGE_1\alpha$-cyclodextrin (58%)[13,15-17]. This is in agreement with Lamprecht, *et al*[38], who reported that PGE_1 (alprostadil) caused fewer side effects than iloprost; on the other hand the latter was effective in a limited number of patients refractory to alprostadil. Moreover Torley, *et al*[15] infused 0.5 ng/kg/min iloprost, less than the conventional dose of 2.0 ng/kg/min, and achieved equal clinical benefits with side effects in only one-third of the patients. Most of our patients considered $PGE_1\alpha$-cyclodextrin more effective than other previously used drugs (nitrates, nifedipine, ketanserin). This is in agreement with the results of a metaanalysis of 6 clinical trials with iloprost, by Watson and Belcher. They reported that about half the patients, who had not had any benefit from nifedipine, ketanserin, α blockers, and other drugs, did gain from iloprost[39].

$PGE_1\alpha$-cyclodextrin infusion did not require special precautions. While the infusion rate of iloprost must be progressively increased from 0.5 ng/kg/min up to a maximum of 2.0 mg/kg/min, in order to evaluate the highest dose tolerated by each patient, $PGE_1\alpha$-cyclodextrin was administered at a fixed rate (20 μg/h) in all the patients from the beginning of the therapy.

The second part of the study assessed whether $PGE_1\alpha$-cyclodextrin affected any of the variables indicative of endothelial cell activation and/or damage in SSc. Several changes of endothelial cell phenotype and functions have been described in SSc and seem to contribute to the typical chronic microvascular injury[40]. Increased expression of adhesion molecules such as ICAM-1 has been identified on

endothelial cells (and fibroblasts) in tissue samples from SSc patients[41,42], particularly where the vessels are surrounded by infiltrating lymphocytes. High levels of the corresponding soluble form (cICAM-1) have been measured in plasma of patients with SSc[23,42,43]. The endothelial injury occurring in SSc also causes derangements of homeostasis between the procoagulant and fibrinolytic properties of endothelial cells[40]. The evidence includes high plasma concentrations of vWF and t-PA resulting from their release from the damaged endothelial cells[25,26].

We measured cICAM-1, vWF, and t-PA as markers of endothelial injury. We also measured TNF-α and sIL-2R, as the former (like IL-1 and interferon-γ) can induce expression of ICAM-1 on endothelial cells[27], and the latter is considered indicative of lymphocyte activation in SSc[28]. In agreement with previous observations[25,26,28,42-44], scleroderma patients had higher plasma levels of sIL-2R, TNF-α, cICAM-1, t-PA, and vWF than healthy controls (Figure 2). Although an analysis of these variables in different subsets of disease (Table 4) was not the main purpose of this study, we found higher levels of TNF-α, sIL-2R, and cICAM-1 in patients with the diffuse form of the disease and in those with lung involvement, in agreement with previous observations[45-47]. Whether increased plasma levels of TNF-α, sIL-2R, and cICAM-1 are specific markers of lung involvement or simply reflect extension of the disease to a larger skin area in "diffuse SSc" and the higher prevalence of lung fibrosis in this subset of patients is at present unknown. We did not, on the other hand, find any correlation with the duration of disease. We could not confirm that in the early phase of the disease plasma levels of sIL-2R, TNF-α, and cICAM-1 were any higher than in the late stages[46,48].

The clinical improvement in SSc patients after PGE_1α-cyclodextrin was accompanied by a significant reduction in the plasma concentrations of cICAM-1 and t-PA. sIL-2R was significantly reduced only after the third cycle of infusion, while vWF and TNF-α levels were slightly, but not significantly reduced after the treatment.

To our knowledge this is the first observation that a drug administered for RP improves variables of activation and/or damage of the endothelium in patients with SSc. Denton, *et al*[49] measured cICAM-1, VCAM-1, and E-selectin at roughly 6 month intervals for more than 3 years in 12 patients with SSc. Plasma concentrations of these adhesion molecules changed significantly within each patient over time, sometimes in relation to changes in clinical conditions. In our study the reduction of cICAM-1 was reproducible, being observed in 23 of 24 patients, making it unlikely that these were fluctuations in the concentrations.

There are several possible explanations of the effect on cICAM-1. PGE_1α-cyclodextrin may downregulate one or more of the cytokines that induce the expression of ICAM-1 on endothelial cells. Della Bella, *et al*[50] found that mononuclear cells from patients with SSc produce less IL-1 and also less TNF-α *in vitro* after patients had been treated with iloprost for one year. In our study, however, TNF-α levels were not reduced after PGE_1α-cyclodextrin; this seems to exclude that the drug acts on cICAM-1 by reducing the TNF-α concentration.

Alternatively, PGE_1α-cyclodextrin may directly downregulate the expression of cICAM-1. Some experimental data with PGE_1 and other prostaglandin derivatives support this. Cicaprost (an analog of PGI_2) and PGE_2 reduce the cytokine mediated expression of ICAM-1 (and VCAM-1) in human vascular and airway smooth muscle cells by increasing intracellular cAMP[51,52]. Although the increase in cAMP does not reduce the TNF-α mediated expression of ICAM-1 in endothelial cells[53], PGE_1 inhibits TNF-α induced T cell adhesion to endothelial cells by selective downmodulation of ICAM-1[54]. In addition, misoprostol, a PGE_1 analog, reduced the expression of ICAM-1 on the vascular endothelium of rat gastric mucosa caused by aspirin or indomethacin[55].

A third possibility is that PGE_1α-cyclodextrin improves the status of the endothelial cell lining by reducing or preventing ischemia reperfusion injury. Cultured endothelial cells express ICAM-1 (both mRNA and protein) after anoxia and reoxygenation[56,57], and in different animal models PGE_1 protects tissues such as heart and liver against ischemia reperfusion injury by reducing leukocyte-endothelial cell adhesion by downmodulating ICAM-1 expression on the endothelial cells[58,59].

If ischemia reperfusion mediated ICAM-1 expression on endothelial cells contributes to the migration of leukocytes to the area of tissue injury and to the perivascular inflammation once the cells are recruited from the circulation, a close control of vasospasm is advisable and may slow the progression of the disease. Veale, *et al*[60] reported that when SSc patients underwent hypoxia, e.g., by application of a tourniquet to the arm, the levels of cICAM-1 rose further. The change in cICAM-1 concentration after a hypoxic stimulus correlated inversely with the skin score, suggesting that in the diffuse form of the disease there is already a submaximal expression and shedding of ICAM-1, but in limited SSc hypoxia can further worsen endothelial function.

The possibility of PGE_1α-cyclodextrin improving endothelial cell function by vasodilating and reducing ischemia reperfusion injury is supported by the finding that the plasma levels of t-PA, another marker of endothelial damage, also decrease after infusion. Other prostanoids seem to have the same effects, as continuous infusion of prostacyclin reduces t-PA and plasminogen activator inhibitor-1 in patients with primary pulmonary hypertension[61]. On the other hand, we found only a slight reduction of plasma levels of vWF after PGE_1α-cyclodextrin. The reason for this is not known. This factor is synthesized by endothelial cells and megakaryocytes and can be stored both in the Wiebel-Palade bodies of endothelial cells and in the alpha granules of platelets. Although evidence from tissue culture and cold challenge to patients with SSc suggests

that endothelial cells are probably the main source of vWF[25], there may also be a release of vWF from platelets in SSc patients[26,62]. PGI_2, which is about 50 times more effective than PGE_1 as a platelet anti-aggregating agent, reduced vWF plasma levels in primary pulmonary hypertension[11,63]. Differences in the kinetics of production and release of t-PA and vWF during the process of endothelial cell repair have also to be considered, as in that study prostacyclin was given for a whole year.

We found that PGE_1α-cyclodextrin significantly reduced the frequency of RP attacks and improved indicators of endothelial cell damage. The restoration of endothelial cell function might explain the prolonged therapeutic effect, which lasts well beyond the infusion. Whether endothelial cell function is exclusively modulated by prostanoids or can be influenced by other vasodilators and whether it is affected by seasonal temperature changes remain to be clarified.

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