EXTENDED REPORT

The myth of pulmonary Raynaud's phenomenon: the contribution of pulmonary arterial vasospasm in patients with systemic sclerosis related pulmonary arterial hypertension

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Objective: To investigate the contribution of cold induced pulmonary vasospasm by peripheral and central cold stimulus in exacerbating pulmonary arterial hypertension (PAH) in patients with systemic sclerosis undergoing cardiac catheterisation.

Methods: In a prospective pilot study, 21 patients with systemic sclerosis and catheter proven PAH had mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), and cardiac output (CO) measured before and after peripheral (hand immersion into cold water at 10–15˚C for two minutes if tolerated) and central (direct cold water at 4˚C injected into the right atrium) cold pressor challenge. Markers of endothelial activation, platelet function, and nitric oxide degradation were measured in blood sampled from the pulmonary artery.

Results: 19 of the patients (mean (SD) age, 56 (4) years; baseline mPAP, 34 (8) mm Hg; PVR, 420 (87) dyne.s.cm^{-5}; CO, 6.4 (1.8) l/min) tolerated cold hand immersion for the maximum two minute duration. All 21 tolerated central cold pressor challenge (three to five injections of 10 ml saline boluses at 4˚C). There was no significant change in haemodynamics after cold challenge by either route of provocation. Levels of endothelin-1, von Willebrand factor, fibrinogen, and 3-nitrotyrosine were raised compared with control values in patients with systemic sclerosis but without PAH, but did not change significantly after peripheral cold challenge.

Conclusions: Pulmonary vasospasm in response to peripheral and centrally administered cold pressor challenge is unlikely to contribute to persistence of pulmonary arterial hypertension in patients with systemic sclerosis.

Digital vasospasm or Raynaud's phenomenon is reported to occur in 90% of patients with systemic sclerosis,[1] being a defining feature of the disorder.[2] Approximately 10–15% of patients with systemic sclerosis also develop pulmonary arterial hypertension[3] which, if uncontrolled, can lead to right ventricular failure and death. Raynaud's phenomenon of the pulmonary vasculature has been proposed as a potential mechanism for the development of pulmonary arterial hypertension (PAH), in both animal models and humans.[4–6] However, several aspects of the mechanism of induction of this proposed "pulmonary Raynaud's phenomenon" remain poorly understood or their existence contested.

In human studies, pulmonary vasospasm has been provoked predominantly by two techniques. Several investigators[7–10] have used digital cold challenge of the peripheries with post-exposure measurement of the carbon monoxide transfer factor (DLCO) or other surrogate markers of pulmonary blood flow, such as radioisotope labelled inert gases. They have implied that a fall in DLCO is evidence of pulmonary Raynaud's phenomenon. Ploysongsang and Foad[7] found an increase in dead space ventilation after a cold pressor test in 13 patients who had digital Raynaud's phenomenon in association with various connective tissue diseases. They concluded that this signified a redistribution of blood flow in the lungs. Vergnon *et al*[8] and Kozielski *et al*[10] found a decrease in early and late DLCO in Raynaud's patients compared with controls. Similarly, Barr and Fahey[9] found a fall in the diffusing capacity of the pulmonary membrane and in the volume of blood in the pulmonary capillaries in eight of 12 patients with digital Raynaud's phenomenon (both primary and secondary) but no such changes in eight controls.

Other investigators have injected cold saline directly into the right atrium[11 12] and simultaneously measured pulmonary artery pressures by cardiac catheterisation. Using this approach, Shuck *et al*[11] and Aalbers *et al*[12] found no significant increase in mean pulmonary artery pressure or pulmonary vascular resistance after digital cold pressor challenge despite an increase in aortic pressure and systemic vascular resistance in nine patients. A further method of provocation, not formally examined in a trial but previously reported,[13–15] was inhalation of cold air with measurement of DLCO in army personnel in extreme temperatures. Again, the investigators found a fall in DLCO with cold exposure, but the studies did not provoke digital Raynaud's phenomenon in their subjects, nor were pulmonary arterial temperatures or blood flow directly measured.

Only one previous study has involved patients with systemic sclerosis and PAH (SScPAH) (diagnosed by echocardiography). These investigators[5] reported a net fall in DLCO by 15% in seven of nine patients undergoing total body cooling to provoke digital Raynaud's phenomenon. Given that patients with systemic sclerosis and early PAH are the

Abbreviations: CCA, calcium channel antagonist; CO, cardiac output; DLCO, transfer factor for carbon monoxide; mPAP, mean pulmonary artery pressure; mRAP, mean right atrial pressure; PAH, pulmonary arterial hypertension; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RV, right ventricle; SScPAH, systemic sclerosis related pulmonary arterial hypertension

most likely to show pulmonary vasospasm, we aimed to assess whether patients had a rise in pulmonary artery pressures or a fall in cardiac output when challenged with cold exposure both peripherally and centrally.

To further investigate mechanisms of pulmonary vascular hyperreactivity, we measured markers of endothelial cell activation from blood sampled directly from a branch of the pulmonary artery. The soluble isoform of endothelin-1 is reported to be increased in patients with systemic sclerosis who have digital Raynaud's phenomenon,[16] and antagonism of its receptor has been employed in therapy.[17] Endothelin-1 levels would be expected to rise in the pulmonary artery after peripheral cold pressor challenge if there was a significant vasospastic contribution to PAH in systemic sclerosis. Measurement of von Willebrand factor and fibrinogen would be useful as well, given reports that they are increased during platelet activation in patients affected by systemic sclerosis and digital Raynaud's phenomenon.[16] 3-Nitrotyrosine, one of the peroxynitrite oxidation products of excess NO, is reported to be raised in states where reactive oxygen species are formed.[18] Changes in concentrations of this peptide would provide an indication of the degree of vasoconstriction induced during cold provocation. The pharmacokinetics of endothelin-1 after acute cold challenge have been established previously. There is a biphasic rise in concentration: an initial increase immediately after cold provocation, followed by a second sustained rise 12 to 24 hours post-provocation.[19] However, the time frame for the rise in von Willebrand factor, fibrinogen, and 3-nitrotyrosine after digital cold provocation remains to be established, though it has been suggested that levels of these peptides rise in a similar pattern to that of endothelin-1.[20 21] We therefore studied changes in both pulmonary haemodynamics and endothelial function in a cohort of patients with systemic sclerosis suspected of having PAH, using cold pressor challenges given both peripherally and centrally.

Our aims in this study were, first, to measure mean pulmonary artery pressure (mPAP), total pulmonary vascular resistance (PVR), and cardiac output (CO) before, during, and after peripheral cold challenge in patients with SScPAH undergoing cardiac catheterisation; second, to assess whether peripheral cold challenge leads to changes in serum vasoactive peptide levels sampled from the pulmonary artery that reflect endothelial activation; and third, to investigate the effect of a central cold challenge by measuring pulmonary haemodynamics at the moment of peak change in pulmonary artery temperature following an injection of 10 ml of cold saline directly into the central circulation.

METHODS

Population

The local ethical practices subcommittee approved the study. Twenty one patients with systemic sclerosis attending the Royal Free Hospital connective tissue disease clinic, London, were studied prospectively between March and May 2002. All patients met the American college of Rheumatology (ACR) criteria[2] for systemic sclerosis. Full informed consent was obtained. None of the patients had digital gangrene or active digital ulceration at the time of the study. The baseline demographic characteristics of the patients under study are shown in table 1.

Table 1 Baseline demographics of 21 patients with systemic sclerosis who had catheter proven pulmonary artery hypertension and who underwent cold hand immersion

Type	No pulmonary fibrosis	Pulmonary fibrosis present
N	7	14
M:F	0:7	2:12
NYHA grade III dyspnoea	4	9
Age (years)	58 (7)	54 (6)
Duration (years)	7 (4)	9 (4)
DLCO (%)	56.2 (3.6)	47.3 (5.1)
TLC (%)	68 (5.3)	57 (9.4)
PASP (mm Hg)	47 (4)	45 (3)
Autoantibody	All ACA+	5 Scl70+

Values are mean (2SD) or n.
ACA, anticentromere antibody; DLCO, % predicted transfer factor for carbon monoxide; F, female; M, male; NYHA, New York Heart Association functional class; PASP, pulmonary artery systolic pressure; TLC, total lung capacity.

Patient selection

Echocardiography and pulmonary function tests are carried out regularly in all patients with systemic sclerosis in our unit as part of routine screening. Pulmonary artery systolic pressure (PASP) of >35 mm Hg on echocardiography was used to screen for patients likely to have pulmonary hypertension. Additional clinical criteria were a DLCO of ⩽40% of predicted or a precipitous fall in DLCO compared with previous values on serial pulmonary function tests, and unexplained dyspnoea.

Fourteen patients were identified as having pulmonary fibrosis if they had extensive (grade III/IV) changes visible on high resolution computed tomography of the thorax and reduced total lung capacity (<70% predicted). These patients were defined as ''fibrotic''. In contrast, seven patients with either no pulmonary fibrosis or minimal fibrosis on high resolution computed tomography (HRCT) (grade I/II) with total lung capacity >70% of predicted were defined as ''non-fibrotic''. Thromboembolic disease was excluded by doing a V/Q scan or spiral contrast computed tomography when clinically indicated. High risk patients identified by the above combination of non-invasive tests and clinical assessment were invited for right heart catheterisation.

All patients met the modified National Institutes of Health criteria[22] for PAH on cardiac catheterisation (mPAP >25 mm Hg at rest or >30 mm Hg on exercise, with pulmonary capillary wedge pressure <14 mm Hg); 14 patients had pulmonary fibrosis with grade III/IV changes on thoracic HRCT as defined by MacDonald *et al*.[23]

Right heart catheterisation and acute vasodilator challenge

Informed consent was obtained before the procedure. A 7 F, triple lumen, balloon tipped, flow directed Swan Ganz pulmonary artery catheter (Baxter Edwards, Irvine, California, USA) was introduced through the right femoral vein using the Seldinger technique under strict aseptic conditions. Serial measurements of central venous pressure, pulmonary artery systolic/diastolic/mean pressures, pulmonary capillary wedge pressures, and cardiac output (measured by the Fick method) were taken. The baseline assessments were repeated at five minute intervals until PVR varied by ±10%. Systemic and mixed venous oxygen saturations were measured at baseline. PVR was calculated as $[mPAP - PCWP]/CO \times 79.92$ dyne.s.cm^{-5}, where PCWP = pulmonary capillary wedge pressure and CO = cardiac output.

Peripheral cold pressor challenge and sample collection

Following completion of the baseline measurements at room temperature (22–25℃), the patient's left hand was fully immersed to wrist level in a cold water bath (10–15℃) for two minutes using the technique suggested by Shuck *et al*.[11] The patient kept the hand in the cold water either for two

minutes or, in case of severe discomfort, until they experienced considerable pain. Immediately before removal of the hand from the water, haemodynamic indices were measured, including mPAP, PVR, and CO. During the recording of PCWP at baseline and following cold challenge, 10 ml of whole blood were collected from the distal port of the thermodilution catheter with the balloon fully inflated and wedged at the most distal pulmonary vessel (diameter <5 mm). This was a sample of blood from a point closest to the pulmonary vasculature, where local markers of endothelial activation have their site of action. Serum and plasma samples were separated by centrifugation within five minutes of collection at 3000 $\times g$ for 10 minutes and aliquots were stored at −40℃ for later analysis.

Central cold pressor challenge

Following this peripheral cold challenge, the patient was given an opportunity to rewarm the hand for a further five minutes. After this recovery period, baseline values of mPAP and PVR were recorded again. The peak change in pulmonary artery temperature was measured after injecting 10 ml of cold saline (4℃) down the right atrial limb of the thermodilution catheter. Measurements of mPAP and PVR were made at the point of maximum fall in pulmonary artery temperature during each bolus injection of cold saline. Thermodilution cardiac output measurements were made using a Siemens Thermocath 2000 cardiac output machine (Siemens Instruments, Bern, Switzerland) and recorded over three to five injections. Vasoactive peptide analyses was not carried out during this part of the study as they had been done previously.

Vasoactive peptide analysis

Commercially available enzyme linked immunosorbent assay (ELISA) test kits were used for endothelin-1 (Cozart Bioscience, Abingdon, UK) and 3-nitrotyrosine (HyCult Biotech, Seattle, Washington, USA). Fibrinogen was measured by a modified Clauss technique using bovine thrombin and a Thromboscreen T400C coagulometer (Pacific Hemostasis, Huntersville, North Carolina, USA). Levels of von Willebrand factor were determined by ELISA using an assay previously described by Herrick *et al*.[24]

Statistical analysis

Statistical analysis was carried out using Student's paired *t* tests to compare baseline haemodynamic measurements with those recorded during the vasospastic phase of provoked Raynaud's phenomenon of the hand. Similar techniques were applied to analyse the haemodynamic data acquired before and after central cold saline injection and the paired samples of vasoactive plasma and serum markers.

Treatment and follow up

None of the patients had been on calcium channel antagonist (CCA) treatment for three months before the cardiac catheterisation. Eleven patients had been on CCAs for treatment of their Raynaud's phenomenon in the past, but as it was ineffective they had discontinued this treatment two years before the present cardiac catheterisation. The other 10 patients had never taken CCAs previously. Eleven patients were on vitamin C and E supplements and seven were on evening primrose oil supplements before the procedure.

After the investigation, all patients were actively followed up in the pulmonary hypertension clinic and managed according to the guidelines outlined by Gibbs *et al*.[25]

RESULTS

Twenty one patients underwent cardiac catheterisation with cold hand immersion during the study period. There was no significant difference in echo estimated tricuspid gradient between patients with and without pulmonary fibrosis (table 1). All patients met the diagnostic criteria for PAH. During the cold pressor challenge, all patients had physical signs of triphasic digital Raynaud's phenomenon. Nineteen of the patients were able to keep their hand immersed in cold water for the maximum two minute duration, and two had to remove their hands after a mean period of 85 seconds. After completion of this phase of the study, there was full resolution of digital vasospasm in all patients on rewarming, with no residual ischaemia. All patients tolerated the central challenge with intrapulmonary cold saline injection. There was no significant rise in heart rate or arterial pressure.

There was no change in haemodynamic variables after peripheral provocation of Raynaud's phenomenon by cold hand immersion (table 2) in the patients with systemic sclerosis with (n = 14) or without (n = 7) pulmonary fibrosis. There was no change in mPAP, PVR, or CO with cold saline injection directly into the pulmonary arteries (table 3). Levels of endothelin-1, fibrinogen, von Willebrand factor, and 3-nitrotyrosine taken from the pulmonary artery catheter were raised above reference range values (obtained from patients with systemic sclerosis but no PAH) in all patients. These values did not change significantly from baseline measurements after peripheral cold challenge (table 4). There was a fall in endothelin-1 and 3-nitrotyrosine levels and a rise in von Willebrand factor and fibrinogen levels but none of these changes was significant.

DISCUSSION

Our results show that there is no significant net increase in mean pulmonary artery pressure in patients with systemic sclerosis with mild to moderate pulmonary arterial hypertension during provocation of digital Raynaud's phenomenon by

Table 2 Haemodynamic variables at baseline and after cold hand immersion in 21 patients with systemic sclerosis and pulmonary arterial hypertension, with and without fibrosis

	Non-fibrotic pulmonary hypertension (n = 7)		Fibrotic pulmonary hypertension (n = 14)	
Variable	Baseline value	After cold challenge	Baseline	After cold challenge
mRAP (mm Hg)	4.6 (3.0)	4.4 (3.0)	5.6 (3.0)	5.5 (3.0)
mPAP (mm Hg)	32 (8)	29.5 (6.0)	44 (6)	44 (6)
RVEDP (mm Hg)	3.6 (1.2)	3.4 (1.1)	4.3 (1.9)	3.9 (1.6)
PVR (dyne.s.cm^{-5})	327 (56)	311 (38)	450 (83)	460 (74)
CO (l/min)	6.3 (1.4)	7.0 (1.2)	4.2 (1.4)	4.1 (1.3)
SVO_2 (%)	69 (6)	71 (5)	56 (4)	54 (4)
MAP (mm Hg)	98 (9)	106 (7)	92 (6)	85 (4)
Heart rate (beats/min)	92 (5)	95 (8)	98 (11)	107 (13)

Values are mean (SD).
There was no significant rise in mPAP in either subgroup
CO, cardiac output; mAP, mean arterial pressure; mPAP, mean pulmonary artery pressure; mRAP, mean right atrial pressure; PVR, pulmonary vascular resistance; RVEDP, right ventricular end diastolic pressure; SVO_2, mixed venous oxygen saturation.

Table 3 Change in mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output from baseline in 21 patients with systemic sclerosis and pulmonary arterial hypertension at maximum temperature change (dT) following a 10 ml bolus of cold saline injected into the proximal pulmonary artery through the right atrial limb of a Swan-Ganz catheter

Patient	mPAP pre-injection (mm Hg)	mPAP post-injection (mm Hg)	Change in PVR at max dT (dyne.s.cm^{-5})	Change of CO from baseline (l/min)	Maximum temp change* (dT)
1	28	27	−4	+0.34	3
2	43	42	−7	+0.42	4
3	36	32	−5	+0.84	3
4	37	39	+6	−0.45	5
5	32	34	+5	−0.51	3
6	33	37	+9	−0.92	3
7	24	23	−4	+0.30	4
8	49	47	−8	+0.96	3
9	26	26	0	+0.02	2
10	36	39	+7	−0.58	3
11	25	27	+5	−0.67	3
12	33	34	+3	−0.35	4
13	30	30	0	−0.02	4
14	34	31	−7	+1.24	2
15	43	46	+6	−1.17	3
16	52	52	0	+0.33	5
17	34	29	−14	+2.40	5
18	33	29	−9	+1.69	4
19	40	42	+6	−0.77	4
20	38	41	+7	−1.31	5
21	45	46	+3	−0.40	3
Mean†	**35.7 (6.2)**	**31.7 (5.9)**	**−1**	**+0.12**	**1.79**

Results for individual patients are mean values for three injections given over a period of five minutes.
*Maximum temperature change recorded on thermodilution CO machine (see text for specification).
†None of the mean indices of haemodynamic change was statistically significant (all $p>0.4$).
CO, cardiac output; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance.

cold water hand immersion. Changes in all three haemodynamic indices were non-significant. This is consistent with the findings of Shuck *et al*.[11] Those investigators also studied pulmonary artery pressures, cardiac output, and pulmonary vascular resistance after cold pressor challenge in nine patients with systemic sclerosis and found no difference in haemodynamics. Furthermore, in our study, no significant change in pulmonary haemodynamics was demonstrable when the cold stimulus was given centrally—a route of provocation of pulmonary vasospasm that has not been investigated previously.

Several techniques have been used in the past to investigate the contribution of pulmonary Raynaud's phenomenon to the development of pulmonary arterial hypertension. These studies have provided conflicting results. Barr and Fahey[9] reported a decrease in lung DLCO in eight of a heterogeneous group of 12 patients with primary and secondary Raynaud's phenomenon. They claimed that the fall in DLCO indicated a reduction in capillary blood volume, thus implying the presence of pulmonary vasospasm. Interestingly, there was no reduction in DLCO in the group with systemic sclerosis and secondary Raynaud's phenomenon—precisely the group in which one would expect the most marked fall. The investigators explained this finding by suggesting that subclinical pulmonary fibrosis could limit the responsiveness of the pulmonary circulation. Furst *et al*[26] found decreased pulmonary perfusion by krypton lung scanning in five of nine patients with systemic sclerosis but without PAH. They claimed that their findings indicated decreased pulmonary blood flow caused by vasospasm. However, their results have not been confirmed in a larger cohort, nor has this technique been used subsequently.

We did not find a significant change in levels of endothelin-1, von Willebrand factor, fibrinogen, or 3-nitrotyrosine after peripheral cold challenge. However, levels were increased compared with reference values from subjects with systemic sclerosis but no PAH.[27] Dziadzio *et al*[16] have previously observed that these peptides are raised in patients with systemic sclerosis who have peripheral Raynaud's phenomenon, as a response to endothelial activation. The lack of a similar response may indicate either that there was no significant pulmonary vasospasm or that these markers do not reflect acute change (within minutes of provocation) in patients who already have raised basal levels. A similar lack of acute elevation was reported by Sakamoto *et al*[28] in blood sampled within 15 minutes of cold pressor challenge, in

Table 4 Change in markers of endothelial activation following peripheral cold challenge

Vasoactive peptide	Baseline	Reference range*	Post cold challenge	p Value (paired *t* test)
Endothelin-1 (fmol/ml)	1.87 (0.13)	0.18 to 1.52	1.83 (0.11)	0.4
vWF (IU/dl)	245 (39)	23 to 177	253 (42)	0.4
Fibrinogen (μmol/l)	3.15 (0.45)	1 to 2.18	3.54 (0.63)	0.5
3-Nitrotyrosine (nM)	610 (80)	200 to 1500	548 (73)	0.6

Values are mean (SD) and range.
*Reference ranges derived from control patients with systemic sclerosis and no pulmonary artery hypertension. Samples taken from peripheral vein in control patients.
vWF, von Willebrand factor.

contrast to Zsmora *et al*[29] who found that endothelin-1 and NO levels reached a peak after 12 to 24 hours of provocation.

Our findings of a lack of immediate change in pulmonary haemodynamics and markers of endothelial activation following cold pressor challenge in patients with catheter proven SScPAH have not been reported previously. Though there is evidence of organ specific vasospasm in the heart[30] and kidneys[31] in systemic sclerosis, this phenomenon appears not to apply to a similar extent to the pulmonary vasculature.

Two thirds of the patients in our study had pulmonary fibrosis. All patients without pulmonary fibrosis were positive for the anticentromere antibody and had a mean pulmonary artery pressure of 32 mm Hg at baseline. Patients with fibrosis had a mean pulmonary artery pressure of 44 mm Hg at baseline, compared with 32 mm Hg in non-fibrotic patients. Interestingly, though it can be argued that the pathogenesis of PAH in these two subgroups may at least be partially different, we found no difference between these two groups in terms of their response to cold challenge. There was no significant change in mPAP, CO, or PVR in the fibrotic subgroup, and in the non-fibrotic subgroup the mPAP fell from 32 to 29.5 mm Hg post cold challenge (NS). Hence, though the fibrotic patients had higher baseline mPAP values, there was no difference in the degree of vasoreactivity to central or peripheral cold challenge between these groups of patients.

Another confounding factor could have been a predominant adrenergic response to the pain induced by cold challenge. Though this aspect was not rigorously investigated, the lack of increase in heart rate, arterial pressure, or systemic vascular resistance suggests that it was not a significant contributor.

Limitations

We did not examine the pulmonary vascular response beyond 10 minutes after cold water hand immersion. It is possible that changes may have occurred later. However, the vasospastic response is usually rapid in the hands, and hence one would expect to find haemodynamic changes in the pulmonary circulation within this time period. Second, we did not have a control group of patients with systemic sclerosis but without PAH. This was not possible because it would have been unethical to carry out invasive cardiac catheterisation in subjects not suspected of having SScPAH. Third, we did not investigate provocation by the inhaled route as logistical limitations put this beyond the scope of our study.

Conclusions

Our initial results suggest that pulmonary vasospasm in response to a cold stimulus that elicits digital vasospasm is unlikely to play a major role in the pathogenesis of pulmonary hypertension in systemic sclerosis. This holds true even when the cold challenge is given directly into the pulmonary arteries. We suggest that the concept of ''pulmonary Raynaud's phenomenon'' does not contribute to the perpetuation of pulmonary hypertension in patients with systemic sclerosis.

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