Endothelial-dependent vasodilation is impaired in patients with systemic sclerosis, as assessed by low dose iontophoresis

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We and others have previously described the non-invasive process of iontophoresis of vasoactive chemicals, in which the chosen ions are driven into the skin by application of a low voltage, in patients with primary Raynaud's phenomenon (PRP) and systemic sclerosis (SSc) (1-6). Microvascular responses to the iontophoresis are quantified by laser Doppler flowmetry. So far results have been somewhat conflicting. In our own studies, forearm (1) and digital (2) skin blood flow responses to the endothelial-dependent vasodilator acetylcholine chloride (ACh), the endothelial-independent vasodilator sodium nitroprusside (NaNP) and the vasoconstrictor adrenaline were similar in patients with PRP, SSc and healthy control subjects. In contrast, Khan *et al.* reported impairment of both endothelial-dependent and independent responses in patients with PRP (4) and impairment of endothelial-dependent vasodilation in patients with SSc (5). La Civita *et al*. reported that both were impaired in SSc (3), while Marasini and Conciato recently reported that the ACh response was impaired in SSc patients (6).

We set out to test the hypothesis that by using shorter periods of iontophoresis of vasodilator chemicals at lower voltages, we would demonstrate impaired vasodilation in SSc patients compared to controls. After applying this new protocol to endothelial-dependent vasodilation and finding this to be impaired in patients with SSc (as detailed below), we then invited patients to attend on a second occasion to examine endothelial-independent responses.

Fifteen patients with SSc (3 male, 12 female; median age 48 years, range 30-55 years) and fourteen healthy control subjects (5 male, 9 female; median age 39 years, range 24-65 years) participated in the ACh part of the study. In the SSc group, five patients had diffuse cutaneous SSc (DCSSc) and 10 had limited cutaneous SSc (LCSSc) (7). 1% ACh (Aldrich, Gillingham) gel was iontophoresed on the dorsum of the index finger proximal phalanx (dominant hand), with blood flow response measured by single probe laser Doppler as previously described (1, 2).

The protocol comprised seven 10-second periods of iontophoresis at increasing doses (30, 40, 50, 60, 70, 85 and 100 microamps) with 60 seconds of blood flow monitoring between each iontophoresis period. This compared to our previous protocol of three 30 s periods at 71 microamps (2). A subset of 8 patients with SSc (3 male, 5 female; median age 48 years, range 42-53 years, 3 DCSSc and 5 LCSSc) and 7 healthy controls (3 male, 4 female; median age 36 years, range 26-45 years) attended on one further occasion 6 to 9 months later when the protocol was repeated, this time using 1% NaNP (David Bull Laboratories PTY Ltd, Victoria, Australia) gel.

Blood flow response to ACh/NaNP for each subject was expressed as area under the blood flow curve (AUC) in perfusion units. seconds, normalised for baseline flux: the AUC for the 60 s baseline monitoring period was extrapolated to cover the entire duration of the protocol (multiplied by 550/60), and then subtracted from the total AUC for the full protocol (550 s). Analysis of variance was used to compare AUC between groups, the AUCs having been previously normalised for baseline blood flow. Estimated differences were also calculated with adjustment for age, sex, smoking and vasodilator treatment by analysis of variance.

Results are shown in Table I. Vasodilation in response to ACh iontophoresis was diminished in the SSc group compared to healthy controls. The estimated deficit was 65.6 (1000 perfusion units.seconds), 95% confidence interval: 3.0 to 128.3 ($p = 0.02$). Although the vasodilatory response to NaNP iontophoresis was 31.4 (95% confidence interval: -93.7, 156.6) lower in the SSc group than the control group, this was not statistically significant ($p = 0.59$). Adjustment for age, sex, smoking and vasodilator treatment produced similar results.

Although numbers of patients were small meaning that our results have to be interpreted with caution, our conclusions support those of Khan and Belch (5) and of Marasina and Conciato (6) – endothelial-dependent, but not endothelial-independent, vasodilation is impaired in SSc. Using lower 'doses' of iontophoresis improves the ability of the iontophoresis technique to detect abnormalities in microvascular function in patients with SSc.

Table I. Mean (95% confidence intervals) of AUC in 1000 perfusion units.seconds, normalised for baseline flux.

	ACh			NaNP		
Controls	(n=14)	169.0	(121.2, 216.8)	(n=7)	137.7	(21.4, 254.0)
All SSc	(n=15)	103.4	(58.3, 148.5)	(n=8)	106.3	(34.0, 178.5)
LCSSc	(n=10)	124.4	(60.8, 188.0)	(n=5)	125.1	(16.4, 233.8)
DCSSc	(n=5)	61.3	(4.5, 118.0)	(n=3)	74.9	(-153.6, 303.3)

M.E. ANDERSON, *MRCP*
T.L. MOORE, *BSc*
S. HOLLIS, *MSc*[1]
S. CLARK, *PhD*
M.I.V. JAYSON, *FRCP*
A.L. HERRICK, *FRCP*

University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford, and [1]Medical Statistics Unit, University of Lancaster, Lancaster, UK.

Address correspondence to: Dr Marina E. Anderson, University of Manchester Rheumatic Diseases Centre, Clinical Sciences Building, Hope Hospital, Eccles Old Road, Salford, M6 8HD, UK.
E-mail: manderso@fs1.ho.man.ac.uk

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