Maximum blood flow and microvascular regulatory responses in systemic sclerosis

H. Gunawardena, N. D. Harris1, C. Carmichael2 and N. J. McHugh

Objectives. To investigate microvascular function using laser Doppler imaging (LDI) following response to hyperaemia, neurovascular reflex and iontophoresis in systemic sclerosis (SSc) in comparison with primary Raynaud’s phenomenon (PRP) and age-matched controls. A secondary aim was to evaluate if SSc patients with a higher Medsger vascular score have lower endothelial responses.

Methods. Twenty patients with SSc, 10 PRP and 17 controls were studied. Patients with SSc were scored using the vascular component of the Medsger severity scale. A baseline LDI scan was performed on the dorsal aspect of both hands. Digital responses were quantified following maximum hyperaemic response (MHR), contralateral vasoconstrictor response (CLVc) and iontophoresis with acetylcholine (Ach)-endothelial dependent and sodium nitroprusside (SNP)-endothelial independent. Mean blood flow was quantified over a standard region of interest.

Results. MHR was lower in SSc patients compared with controls (P < 0.001). A similar trend was seen when comparing SSc with PRP although this did not reach significance (P = 0.07). CLVc and Ach/MHR were lower in SSc vs PRP (P < 0.05) and controls (P < 0.001). No difference was observed in MHR, CLVc and Ach/MHR between PRP and controls. Overall, SNP/MHR was similar in all the three groups. SSc patients with a higher Medsger vascular score had lower endothelial-dependent (P < 0.01) and independent (P < 0.05) responses.

Conclusion. SSc patients have abnormal microvascular regulatory responses compared with PRP and controls. This study also suggests that the degree of endothelial dysfunction may be related to the degree of peripheral vascular involvement.

Key words: Endothelin, Microvascular, Laser Doppler imaging, Raynaud’s phenomenon, Systemic sclerosis.

Introduction

Systemic sclerosis (SSc) is an autoimmune disorder characterized through microvascular dysfunction secondary to a number of factors including endothelial damage, over-expression of specific adhesion molecules and perivascular inflammatory cell infiltration [2–5]. In SSc, pathological changes are observed not just in affected skin but also normal skin, indicating early changes in endothelial function [6]. Markers of endothelial activation including endothelial leucocyte-adherence molecule type 1 [7], vascular endothelial growth factor, soluble vascular cell adhesion molecule-1, endothelin-1 (ET-1), IL-6 and IL-10 are all increased in SSc [8–10]. In particular, the balance of vasoactive peptides (ET-1, nitric oxide and prostacyclin) is disturbed and this combined with pro-inflammatory mediators affects vascular integrity and components of the extra-cellular matrix [11]. In addition to changes in endothelial cell function, microvascular disease in SSc is also characterized by abnormal neurovascular control secondary to neuronal damage [12, 13]. The combined effect of all these factors leads to changes in vascular tone and structure with eventual fibro-occlusion of the microvasculature. This pathological hallmark lesion is implicated in a number of clinical manifestations of SSc including Raynaud’s phenomenon, digital ulceration, pulmonary arterial hypertension (PHT) and hypertensive renal crisis.

There is a need for accurate and sensitive techniques to quantify microvascular disease in SSc. Laser Doppler imaging (LDI) is a tool to measure cutaneous microvascular flow and previous studies have shown how laser Doppler can demonstrate abnormalities in vascular function in patients with SSc [14–20]. Early detection of microvascular changes and the relationship with disease progression or severity may provide further insights into the pathophysiology of SSc, with clinical implications in terms of disease monitoring. In this study, we have developed an LDI protocol to measure baseline microvascular flow and then assess changes in tissue perfusion following both a physiological challenge [maximum hyperaemic response (MHR) and neurovascular regulatory response] and pharmacological challenge (iontophoresis: endothelium-dependent and -independent response). The primary aim was to compare microvascular regulatory responses in SSc patients with primary Raynaud’s phenomenon (PRP) and age-matched control subjects. In addition, we have evaluated how digital microvascular responses relate to clinical disease based on the Medsger peripheral vascular severity score [21].

Patients and methods

Patients

Patients currently attending our institution were invited to participate in the study and positive respondents were recruited including 20 patients fulfilling the American College of Rheumatology (formerly, the American Rheumatology Association) preliminary criteria for the classification of SSc [22]. Ten patients with PRP (no clinical or serological evidence of connective tissue disease over 2 yrs) and 17 healthy controls were also recruited. Patients continued their regular medications including specific oral vasodilator therapy. The vascular domain of the Medsger SSc disease severity score was performed prior to LDI assessment [21]. In summary, the score scales patients from 0 to 4 depending on clinical peripheral vascular features. Patients with a history of Raynaud’s scored 1 (mild), digital pitting scars scored 2 (moderate), digital ulcerations scored 3 (severe) and those with digital gangrene scored 4 (end-stage). All patients were screened for isolated pulmonary hypertension (PHT) with lung function and echocardiography. Where indicated a right heart catheter was performed.
The study was approved by the Bath Local Ethics Committee and all patients and controls gave informed written consent. Exclusion criteria included those unable to give informed consent, subjects with a history of uncontrolled systemic hypertension, hyperlipidaemia, cardiac failure, hepatic failure, diabetics, smokers and pregnant or lactating women.

**LDI protocol**

Scanning red LDI-2 (Moor Instruments Ltd, Axmister, Devon) was performed after resting the subject in a temperature-controlled room at 23°C for 20 min. A baseline scan of both hands (dorsal aspect of both sets of fingers) was initially performed including baseline flux of the individual fingers (index, middle and ring) to establish the degree of variability between sites.

To assess microvascular responses, the following procedures were applied. The dorsal aspect of the ring finger of the right hand was heated at 44°C by application of a Peltier temperature-controlled sprung metal plate (VU Instruments, Amsterdam) for 6 min to elicit a MHR. Serial LDI of the heated area was then performed over 10 min (maximum of eight scans) to determine the maximum blood flow quantified in LDI flux perfusion units (Fpu). Single spot laser Doppler flowmetry was performed over the right middle finger nailbed in response to immersion of the (Fpu). Single spot laser Doppler flowmetry was performed over the right middle finger nailbed in response to immersion of the (Fpu).

**Results**

Table 1 summarizes the main features of the three study groups. Patients in the PRP group were younger but SSc patients and controls were age matched. Duration of Raynaud’s phenomenon was similar between SSc and PRP patients. Three patients had diffuse cutaneous SSc (all had a modified Rodnan skin score of >14) and 17 limited cutaneous SSc (all had a Rodnan skin score <14) [23, 24]. There were no significant differences in microvascular responses between disease subtypes, although the number of patients assessed with diffuse disease was small. Eleven patients scored 1 on the Medsger vascular scale, seven patients scored 2 and two patients scored 3. In the SSc group, 10 patients were on calcium channel blockers (CCB) and three on ACE inhibitors for treatment of their Raynaud’s phenomenon. Two patients with PRP were on a CCB. No patient had received intra-venous prostanooids within 6 months of their LDI study or were on endothelial receptor antagonists.

Following acclimatization, the mean blood flow was analysed individually between the index, middle and ring fingers to assess the degree of variability of baseline flow. There was no significant difference in baseline flux between fingers in any of the subject groups (supplementary data are available at Rheumatology Online). In addition, there was no difference in overall baseline flow between hands in the SSc patients (data not shown).

Figure 1 illustrates overall laser Doppler protocol results. MHR was significantly lower in SSc vs controls (P < 0.001) and lower in SSc compared with PRP (P = 0.07). CLVc percentage decrease was found to be significantly lower in patients with SSc vs PRP (P < 0.05) and controls (P < 0.001). Endothelial-dependent (Ach) response was significantly lower in the SSc group compared with PRP (P < 0.01) and controls (P < 0.001). No significant difference was observed in MHR, CLVc, Ach/MHR between PRP and the control group. Endothelial-independent (SNP) response was similar in all three groups. There was no significant difference in MHR, CLVc or vasodilator responses in SSc patients on vasoactive medication compared with those not on medications and so these results have not been reported. The current during iontophoresis was standardized and the voltage within the range of the microcirculatory controller for all subjects.

No correlation was observed between disease duration and microvascular responses (data not shown). However, SSc patients with a Medsger peripheral vascular score ≥2 had a significantly lower Ach/MHR (P < 0.01) and SNP/MHR response (P < 0.05) in comparison the rest of the SSc group who scored 1 (Table 2). CLVc percentage decrease was also lower in those with higher variability in baseline flow.

**Statistical analysis**

Statistics were conducted using SPSS for Windows (version 12) software. All data were tested to see if assumptions of normality were met. The assumptions of normality cannot be assumed within the data set particularly due to the small sample size, therefore non-parametric tests were used throughout. To compare baseline flow variability between individual fingers and hands, Friedman’s test and the Wilcoxon-signed rank test were used, respectively. The Kruskal–Wallis test was used when comparing the three study groups in terms of their MHR, CLVc and iontophoresis responses. When the Kruskal–Wallis test showed a statistically significant result the Mann–Whitney U-test was used to test individual differences between two individual study groups. Any dichotomized data were also analysed using the Mann–Whitney test. Spearman’s rank order correlation was used to test for an association between disease duration and vascular responses. When comparing results, median values and interquartile ranges (IQR) were used as the data are non-parametric. P-values <0.05 were considered significant.

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<th>PRP (n=10)</th>
<th>Controls (n=17)</th>
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<td>Age</td>
<td>56 (44, 62)</td>
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<td>Duration of symptoms</td>
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variable results and this may have been due to heterogeneity of the area of skin studied [14–19]. Scanning LDI has the advantage of analysing blood flow over a larger surface area which is more reproducible [20, 25] and physiological day-to-day variability of iontophoresis has been previously assessed [25]. Other potential variables include current density, voltage effects and the type of solution used for iontophoresis [25−27]. The choice of solution mixed with Ach or SNP has been shown not to affect the voltage required for iontophoretic transfer [27]. In our study, we have used a large surface area combined with low-current density and the application of current through distilled water alone did not produce vasodilation (data not shown).

The potential disadvantage of previous laser Doppler studies is the lack of a biological reference. Baseline blood flow is commonly used but is quite variable even in controlled conditions and we feel is not a good reference point. In this study, we have used MHR as a biological reference (maximum vasodilation is achieved using a 44°C stimulus) and expressed maximum vasodilation with Ach and SNP against MHR, in order to improve reproducibility. In a previous pilot study, we have demonstrated good reproducibility of the technique [28], and thermal hyperaemic response was also assessed by another group [29]. With this protocol, we assess iontophoretic response at one site in relation to MHR on an adjacent finger. However, baseline flow between the three fingers used (and between hands) prior to the microvascular challenges did not show any significant variability in any of the subject groups.

MHR in itself is a useful marker of overall microvascular function. Vasodilation secondary to hyperaemia is mediated through an axonal reflex and a nitric oxide-dependent response [30, 31]. Recent studies have determined ‘thermal hyperaemia’ in patients with SSc compared with PRP and controls [29, 32]. SSc patients showed a blunted peak response and our data have demonstrated similar findings with lower MHR in the SSc group. We have also demonstrated a significantly lower CLVc in SSc compared with PRP and controls. The CLVc is a neurovascular reflex which measures sympathetic function. In normal subjects, when the ipsilateral hand is placed in cold water, vasoconstriction occurs in the contralateral warm hand via a central mechanism and this reflex is controlled primarily through efferent preganglionic myelinated axonal fibres [33]. Structural damage affects myelinated and unmyelinated nerve fibres in both diffuse cutaneous SSc and limited cutaneous SSc contributing to vascular dysfunction [12–13], and we have demonstrated a blunted contralateral response in SSc patients. This damage to parasym pathetic nerves may be related to endothelial cell injury leading to axonal damage.

Our study shows Ach endothelial-dependent vasodilation is impaired at an early stage in SSc, secondary to loss of vasodilator mediators in particular nitric oxide. Previous studies have shown SNP endothelial-independent response is also impaired [16, 20]. Overall, we have demonstrated similar SNP responses in our SSc, PRP patients and controls as shown in a previous study [18]. There was no correlation with any of the measures and disease duration. However, when the patients were stratified based on the Medsger peripheral vascular score those SSc patients with more severe disease had a significantly lower Ach endothelial-dependent response and SNP endothelial-independent response compared with those with Raynaud’s alone. Therefore, it appears that disease severity in terms of peripheral vascular manifestations is associated with a defect in endothelium-dependent vasodilation and endothelial-independent smooth muscle dilation. In this study, some patients continued on their vasoactive treatments. However, there was no significant difference in microvascular responses when comparing SSc patients on vaso dilators and those not on treatments. In addition, drugs may influence vascular tone but are unlikely to have a significant effect on endothelial function or microvascular structure.
Another question is whether patients with organ involvement in particular PHT have more severe microvascular changes that can be detected using LDI. Certainly, markers of endothelial damage are higher in SSC compared with controls [6–10, 34] and further elevated in those with systemic organ involvement [8, 35]. It is well established that endothelial cell dysfunction plays a critical role in the pathogenesis of digital ulceration and isolated PHT. Of interest, the one patient with PHT had the lowest endothelial responses out of the entire group. These preliminary results appear promising and further work needs to be done to see if microvascular assessment using LDI can be used as a marker of overall disease severity.

In conclusion, patients with SSC have abnormal microvascular responses compared with patients with PRP and controls. This study confirms previous work that shows microvascular assessment may be a useful method of investigating patients who present with Raynaud’s phenomenon. This study also suggests that the degree of microvascular dysfunction may be related to disease severity, particularly digital vascular manifestations. Further longitudinal studies are required to evaluate how endothelial cell function changes in relation to other markers of disease progression and in response to potential disease modifying treatments.

### Rheumatology key messages

- SSC patients have abnormal microvascular responses compared with patients with PRP and normal subjects.
- Microvascular dysfunction measured using LDI relates to the severity of digital vascular disease.

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### References