Concise Report

Microvascular responses following digital thermal hyperaemia and iontophoresis measured by laser Doppler imaging in idiopathic inflammatory myopathy

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

Objective. To measure microvascular function using laser Doppler imaging following digital hyperaemia and iontophoresis of vasoactive substances in patients with idiopathic inflammatory myopathy (IIM).

Methods. Fifteen patients with IIM including eight patients with dermatomyositis (DM) and seven patients with polymyositis (PM) were studied. Fifteen age-matched normal controls were also recruited. Scanning red laser Doppler imaging (LDI) was performed after resting the subject in a temperature controlled room at 23°C for 20 min. An initial LDI scan was performed to assess baseline blood flow. Digital microvascular responses were quantified following a maximum hyperaemic response (MHR) and iontophoresis with endothelial dependent acetylcholine (Ach) and endothelial independent sodium nitroprusside (SNP). Maximum vasodilation following iontophoresis was expressed as a percentage of the MHR.

Results. All subjects were age matched, and the duration of disease was similar between the IIM patients. There was no significant difference in baseline blood flow when comparing the three study groups. There was no significant difference in MHR or SNP/MHR when comparing DM or PM with controls. However, Ach/MHR was significantly lower in both the DM and PM group compared with controls (both \( P < 0.01 \)). There was no significant difference in any of the microvascular responses when comparing patients with DM directly with PM.

Conclusion. This is the first study to evaluate microvascular responses using LDI in patients with IIM. We have demonstrated that patients with DM have abnormal endothelial dependent mediated vasodilation and the same abnormality is present in patients with PM.

Key words: Myositis, Laser Doppler imaging, Microvascular, Endothelial function.

Introduction

The idiopathic inflammatory myopathies (IIM); dermatomyositis (DM) and polymyositis (PM) are heterogeneous conditions characterized by varying degrees of muscle inflammation, skin disease and internal organ involvement [1]. They are disorders driven by either humoral or cellular autoimmunity. In DM, immune responses primarily target the microvascular endothelium and activation of the complement cascade leads to endothelial damage. Inflammatory lesions are predominately perivascular and characteristic pathological changes are seen in both endomyial and dermal capillaries [1–4].

In comparison to DM, microvascular disease appears to play little role in the pathogenesis of PM. In PM, myocytotoxicity is mediated by cell mediated immune responses where CD8 positive T cells are primed to recognize muscle autoantigens leading to an inflammatory cell infiltrate in the muscle fascicles [1]. However, adhesion molecules involved in T-cell transport and cytokine signalling are expressed in endothelial cells and this suggests that activation of the endothelium appears to be involved in the inflammatory process of PM as well as DM [5–6]. Markers of endothelial activation including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and anti-endothelial cell autoantibodies are up-regulated in PM and DM. This suggests that endothelial dysfunction is likely to occur in both subtypes of IIM [6–8].

Clinically, changes in microvascular structure are evident particularly in DM where patients can develop abnormal dilated capillary nailfolds. Furthermore, Raynaud’s phenomenon (RP) is a common symptom in IIM particularly in the subset of patients with anti-synthetase syndrome (ASS). Although not all patients complain of RP, abnormalities in digital vascular tone and structure may still be present. Microvascular blood flow can be assessed using laser Doppler imaging (LDI). This non-invasive technique has been used primarily as a research tool in rheumatological disorders, in particular systemic sclerosis (SSc) [9–11]. Using a standardized LDI protocol, vasodilatory response can be assessed following a physiological challenge (thermal hyperaemic response) and a pharmacological stimulus (iontophoresis). The aim of this study was to quantify the degree of microvascular dysfunction in DM and to see if there was evidence of abnormal endothelial function in patients with PM.

Patients and methods

Patients

Fifteen patients attending our centre with a diagnosis of IIM were recruited for this study. The diagnosis of probable or definite DM or PM was based on the Bohan and Peter criteria [12]. Clinical features including baseline characteristics, a history of RP and autoantibody profile were recorded. Fifteen healthy age-matched controls with no history of RP were also recruited. Subjects were asked to omit any specific vasodilatory medication on the morning of the study. The study was approved by the Bath Local Ethics Committee and all subjects gave informed written consent. Exclusion criteria included those with a history of uncontrolled systemic hypertension, hyperlipidaemia, ischaemic heart disease, renal or hepatic failure, diabetics and smokers.

LDI protocol

This has been described previously [11], briefly prior to each study session the LDI was recalibrated using a flux standard supplied with LDI. Each subject was acclimatized in a temperature controlled darkened room at 23°C for 20 min and then scanning red LDI-2 (Moor Instruments Ltd, Axminster, Devon)

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was performed. The distance of the scanner to the subject was standardized and mean blood flow was quantified in flux perfusion units (Fpu) using inbuilt image analysis software over the defined region of interest (ROI). The ROI was constant within the range of 0.4–0.6 cm² (area between the proximal interphalangeal and distal interphalangeal joint) between subjects. The ROI between serial scans in the individual subjects was the same for data analysis.

An initial scan of baseline flux of the individual fingers (index, middle, and ring) was performed to establish the degree of variability between sites. The maximum hyperaemic response (MHR) of the dorsal aspect of the ring finger of the right hand was evaluated following heating at 44 °C for 6 min by application of a temperature control plate [11]. Serial LDIs of the heated area was then performed over 10 min (maximum of eight scans) to determine the maximum blood flow. Iontophoresis was then performed to assess the maximum nitric oxide dependent and independent vasodilator response. The solutions for iontophoresis were made up using 2% acetylcholine chloride (Ach) (Sigma) and 5% sodium nitroprusside (SNP) (50 mg powder for infusion, Faulding DBL) [11]. The dorsal aspect (the region between the distal interphalangeal and proximal interphalangeal joint) of the index finger was placed in the Ach chamber and the middle finger into the SNP chamber. A 250 microamp current was used to iontophoresis, to determine maximum perfusion units (Fpu) using inbuilt image analysis software over the defined region of interest (ROI). The ROI was constant for data analysis.

Variability between individual fingers within each study group or when compared with baseline flow of the individual fingers (index, middle, and ring) was performed to establish the degree of variability between subjects. The maximum hyperaemic response (MHR) of the dorsal aspect of the ring finger of the right hand was evaluated following heating at 44 °C for 6 min by application of a temperature control plate [11]. Serial LDIs of the heated area was then performed over 10 min (maximum of eight scans) to determine the maximum blood flow. Iontophoresis was then performed to assess the maximum nitric oxide dependent and independent vasodilator response. The solutions for iontophoresis were made up using 2% acetylcholine chloride (Ach) (Sigma) and 5% sodium nitroprusside (SNP) (50 mg powder for infusion, Faulding DBL) [11]. The dorsal aspect (the region between the distal interphalangeal and proximal interphalangeal joint) of the index finger was placed in the Ach chamber and the middle finger into the SNP chamber. A 250 microamp current was applied for three minutes, using Moor Instruments Iontophoresis controller and both drugs were iontophoresed simultaneously. LDI was performed at baseline and then again at 1 min intervals after iontophoresis, to determine maximum perfusion response to Ach and SNP. The maximum vasodilation to Ach and SNP was recorded, and then expressed as a percentage of the MHR.

Statistical analysis
Statistics were conducted using SPSS for Windows (version 12) software. The data was non-parametric, therefore, the following tests were used. Friedman’s test was used to compare baseline flow variability between individual fingers. The Kruskal–Wallis test was used when comparing the three study groups in terms of MHR and iontophoresis responses and the Mann–Whitney U-test was used to test individual differences. Spearman’s rank test was used to test correlation with baseline characteristics and microvascular responses. Results are expressed as median values with inter-quartile ranges (IQR). P-values <0.05 were considered significant.

Results
The baseline characteristics are summarized in Table 1. Eight patients had an established diagnosis of DM and seven patients had PM. All subjects were age matched and the duration of disease was similar between the IIM patients. Six out of 15 IIM patients had a history of RP with a higher frequency in the PM group (P < 0.05). Five PM patients had anti-synthetase autoantibodies and one was positive for the anti-SRP autoantibody. Within the DM group, three patients had known myositis autoantibodies including anti-Mi-2 and anti-PM-Scl. Four DM patients had novel autoantibodies; two precipitated autoantibodies to a 140 kDa protein (unpublished observation) and two had anti-SUMO-E1 autoantibodies [13]. Two of the 15 IIM patients had active disease at the time of their LDI study based on their current clinical features, raised inflammatory markers and creatinine kinase.

There was no significant difference in baseline flow (Fpu) between individual fingers within each study group or when comparing fingers between groups (see supplementary data table). Figure 1 illustrates the overall results comparing MHR and iontophoresis responses between DM, PM and control subjects. There was no significant difference in MHR or SNP/MHR when comparing DM or PM with controls. However, Ach/MHR was significantly lower in both the DM and PM group compared with controls (both P < 0.01). There was no significant difference in any of the microvascular responses when comparing patients with DM directly with PM. In addition, there was no significant difference in microvascular responses in those IIM patients with a history of RP compared with those without (data not shown). The two patients with active disease at the time of the study had a lower median MHR, Ach/MHR and SNP/MHR response in comparison with the rest of the IIM group, although this was not statistically significant (data not shown). There was no correlation with disease duration or creatinine kinase level at disease onset and microvascular responses.

Discussion
Scanning LDI is a non-invasive technique that can measure changes in the skin microcirculation. It has been used primarily as a research tool in rheumatological conditions, in particular SSc. Several studies have shown abnormal endothelial function using a variety of microvascular responses that can be quantified by LDI [9–11]. Similar changes may be expected in patients with IIM.
particularly in DM where the primary antigenic target is the vascular endothelium [1–7]. Markers of endothelial cell activation are also described in PM, and so changes in endothelial microvascular function may be measurable in both subtypes of IIM [5, 7–8].

Using a standardized protocol, we have measured changes in blood flow using LDI following a digital hyperaemic challenge and iontophoresis. Baseline scans demonstrated there was no significant variability in blood flow between individual fingers within groups. This is important as we evaluate maximum vasodilation to Ach and SNP against the MHR. Furthermore, baseline digital blood flow was similar in patients with DM and PM when compared with controls and this is an interesting observation as one might expect lower perfusion in patients with IIM. The MHR is a useful marker of microvascular function in which the initial peak of vasodilation is mediated through an axonal reflex [14, 15]. The MHR was similar between all three groups demonstrating a preserved axonal reflex in IIM. However, there was a trend towards a lower MHR in the PM group compared with DM group, although not significant, this maybe explained by a higher frequency of RP in the PM group.

There was no significant difference in endothelial independent response (SNP/MHR) between IIM patients and controls. This suggests that overall microvascular structure is maintained with normal smooth muscle induced vasodilation; in contrast, these responses have been reported to be abnormal in patients with SSc [9–11]. However, we have demonstrated an abnormal endothelial dependent response in both DM and PM patients compared with normal controls. Of interest, the two patients who had clinical features of active disease at the time of study had lower iontophoresis responses compared with the overall IIM group, which may suggest disease activity is associated with a greater degree of microvascular dysfunction. However, such an observation needs to be interpreted with caution as there were a small number of patients with active disease. In this study, we did not compare subjects with primary RP (PRP) directly with patients with IIM. However, Anderson et al. [10] and a study from our group [11] (using the same LDI protocol) have shown endothelial microvascular responses in RP are similar to normal controls.

One of the concerns about measuring microvascular responses using this technique is reproducibility. Kubli et al. [16] have demonstrated good day-to-day reproducibility. In preliminary work, we have found that by comparing iontophoresis responses against a biological reference response (MHR) can further improve reproducibility [17]. Flow-mediated brachial artery dilatation has been used to assess macrovascular function in patients with secondary RP [18]. A previous study has shown good correlation between laser Doppler measures of skin vasodilation and brachial artery ultrasound measurements [19]. To our knowledge, there are no published studies evaluating thermographic imaging in patients with IIM. Clark et al. [20] have shown in RP (mostly SSc patients) thermographic and laser Doppler results correlate poorly. More recently, calculation of the thermographic parameter, the ‘distal-dorsal difference’ was shown to differentiate between PRP and SRP (SSc and undifferentiated connective tissue disease) [21]. Using a combination of these techniques in IIM and other connective tissue diseases may provide further insights into vascular function.

In summary, this is the first study to evaluate microvascular responses using LDI specifically in IIM. A previous study has investigated endothelial function using laser Doppler flowmetry in response to reactive hyperaemia in 20 patients with various connective tissue diseases, including one patient with PM, but none with DM. The overall results were shown and the responses for the PM patient were not given [18]. We have demonstrated that patients with DM have abnormal endothelial dependent mediated vasodilation and the same abnormality is present in patients with PM. This raises the question of whether cutaneous microvascular responses are abnormal in patients with other connective tissue or inflammatory joint diseases. A recent study has demonstrated endothelial dysfunction using LDI following iontophoresis in eight patients with rheumatoid arthritis, although smokers were not excluded [22]. In contrast, we have observed normal endothelial responses in a group of patients with systemic lupus erythematosus (unpublished observation). More work is required to assess microvascular responses in a larger cohort of IIM patients as well as other connective tissue diseases. Further investigation comparing responses in active disease and how this changes in response to specific immunomodulatory therapies is of major interest.

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References


Rheumatology key messages

- Laser Doppler imaging following digital hyperaemia and iontophoresis can quantify microvascular responses in patients with idiopathic inflammatory myopathy.
- Endothelial dependent vasodilation is impaired in patients with dermatomyositis and polymyositis.


