Digital vascular response to topical glyceryl trinitrate, as measured by laser Doppler imaging, in primary Raynaud’s phenomenon and systemic sclerosis

M. E. Anderson, T. L. Moore, S. Hollis¹, M. I. V. Jayson, T. A. King² and A. L. Herrick

University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford M6 8HD, ¹Medical Statistics Unit, University of Lancaster, Lancaster LA1 4YF and ²Laser Photonics, Department of Physics and Astronomy, University of Manchester, Manchester M13 9PL, UK

Abstract

Objective. To investigate digital microvascular responses to topical glyceryl trinitrate (GTN) in patients with primary Raynaud’s phenomenon (PRP), limited cutaneous systemic sclerosis (LCSSc) and healthy control subjects, using laser Doppler imaging.

Methods. Ten patients with PRP, 13 with LCSSc and 10 control subjects were studied. Baseline skin microvascular blood flow of the dorsum of the index, middle and ring fingers of the non-dominant hand was measured using scanning laser Doppler imaging. After the initial image, 2% GTN ointment was rubbed on the dorsum of one finger for 1 min; placebo ointment was rubbed on the dorsum of a second finger for 1 min, and the third finger remained untreated. Further laser Doppler scanning of these three fingers was conducted immediately, 10 and 20 min after ointment application.

Results. There was increased blood flow response to placebo compared with no treatment (P < 0.001) and to GTN compared with placebo (P = 0.004). The change in blood flow over time differed significantly between placebo and GTN (P < 0.001), but not between placebo and no ointment application: blood flow increased with GTN and decreased with placebo/no treatment at 10 and 20 min. There were no differences in initial baseline blood flow or response between the subject groups.

Conclusions. An exogenous supply of nitric oxide by topical GTN ointment causes local endothelial-independent vasodilatory responses in PRP, LCSSc patients and control subjects. As well as demonstrating the effectiveness of topical GTN in patients with PRP and LCSSc, this study illustrates the ability of laser Doppler imaging to quantify local vasodilatory effects.

KEY WORDS: Topical glyceryl trinitrate, Primary Raynaud’s phenomenon, Limited cutaneous systemic sclerosis, Doppler imaging, Digital microvascular responses.

The treatment of Raynaud’s phenomenon is currently unsatisfactory, both in patients with primary (idiopathic) Raynaud’s phenomenon (PRP) and in patients with underlying connective tissue disease such as systemic sclerosis (SSc). This is partly due to the fact that the pathophysiology of Raynaud’s phenomenon is poorly understood [1], limiting the development of effective treatment strategies. This pathophysiology is likely to differ between primary and secondary Raynaud’s because structural microvascular disease, one of the hallmarks of SSc [2], does not occur in PRP, although minor increases in capillary dimensions may be seen in some PRP patients [3]. Although many different vasodilators have been recommended for Raynaud’s phenomenon, a significant number of patients do not continue on these, either because the medication is ineffective or because it causes intolerable side-effects such as flushing or headaches.

Glyceryl trinitrate (GTN) has been recommended for the treatment of Raynaud’s phenomenon. GTN is a nitric oxide donor and thus its vasodilatory response is endothelial independent and can therefore occur in patients with endothelial injury. This is an important consideration in patients with SSc in whom endothelial

Correspondence to: M. E. Anderson.
damage is well recognized, even in patients with early disease [4]. Nitric oxide donation (as tested by iontophoresis of sodium nitroprusside) results in increased digital microcirculatory flow in patients with SSc and PRP [5]. Matucci-Cerinic et al. [6] reported how patients with SSc vasodilated in response to intravenous GTN, although the response did become blunted with disease progression. However, in a controlled clinical trial of GTN (administered as a patch) in patients with PRP progression, the response did become blunted with disease progression. What is required is an effective vasodilator without significant systemic adverse effects. We have, therefore, investigated the acute effects of topical GTN applied locally to the fingers.

Patients and methods

Patients

Ten patients with PRP (four male, six female; median age 38 yr, range 26–52 yr), 13 patients with limited cutaneous SSc (LCSSc; two male, 11 female; median age 47 yr, range 37–65 yr), as defined by LeRoy et al. [8] and 10 healthy control subjects (four male, six female; median age 35 yr, range 28–63 yr) were studied. The patients in the PRP group all had Raynaud’s phenomenon for at least 2 yr, with neither clinical nor serological evidence of connective tissue disease. Of the patients with LCSSc, one patient had no skin thickening of the fingers (skin involvement of toes only), five patients had mild involvement (involved, but able to pinch) of the skin of the tested finger, six had moderate involvement (unable to pinch, able to move), and one patient had severe involvement (unable to move). In all of these patients, the skin involvement of all fingers of both hands was similar, with no oedema or atrophy at studied sites. All patients with LCSSc had been diagnosed a median of 5 yr (range 1–18 yr) previously. One PRP patient, two LCSSc patients and two control subjects were daily smokers (more than one cigarette per day), whilst two LCSSc patients smoked a few cigarettes each week (less than one cigarette per day). Subjects normally on vasodilator therapy discontinued treatment for the 2 weeks prior to the study. All subjects were instructed to avoid caffeine ingestion and asked not to smoke on the day of the test. Written consent to participation in the study was obtained from each subject and the study was approved by Salford and Trafford Ethics Committee.

Methods

After acclimatization at 23°C for 20 min, baseline skin microvascular blood flow of the dorsum of the middle three fingers (index, middle and ring) of the non-dominant hand was measured using scanning laser Doppler imaging, which uses a helium–neon laser operating at 633 nm (Moor Instruments Ltd, Axminster, UK). The patients wore safety goggles at all times during the experiment and removed all rings from their fingers, if possible.

After the initial image or ‘flux map’:

1. 2% GTN ointment (0.5°Percutol, Dominion Pharma Ltd, Surrey, UK) was rubbed on the dorsum of one finger for 1 min;
2. placebo (petroleum jelly; same as Percutol in colour, texture, odour and absorption) was rubbed on the dorsum of a second finger for 1 min;
3. no ointment was applied to the third finger, in order to allow any systemic effect of the GTN to be identified.

The fingers allocated to the application of GTN, the application of placebo and no application were rotated in the same pre-set order on successive patients. Patient appointments were randomly allocated.

Further laser Doppler scanning of these three fingers was conducted immediately, 10 and 20 min after application of GTN/placebo.

From every laser Doppler image, the whole of each of the three fingers studied was outlined from the base of the proximal phalanx up to and including the nailbed, and the mean cutaneous blood flow of the outlined area for each digit was derived (MoorLDI V3.0 software). The blood flow flux is given in arbitrary perfusion units (PU) with respect to a calibration standard for the MoorLDI scanner. The mean blood flow immediately, 10 and 20 min after application of ointment was expressed as a percentage of the baseline mean blood flow for each finger.

All subjects were asked whether or not they experienced any side-effects.

Statistical analysis

The ratio of blood flow response to baseline blood flow was log-transformed to achieve normality. Using SPSS for Windows, repeated measures ANOVA was applied to the transformed data to examine the effects of time, patient group and treatment.

Results

None of the participants in the study experienced any adverse effects. The results of one patient with PRP were not analysed as they were not of sufficient quality.

Figure 1 shows an example of flux maps for one LCSSc patient (a) before and (b) 20 min after application of GTN/placebo.

The geometric mean (95% confidence intervals) of baseline blood flow across all three fingers was 141 (108, 186) PU for the control group, 105 (58, 189) PU for the PRP group and 135 (90, 201) PU for the LCSSc group. There were no differences in baseline blood flow between the three groups ($P = 0.47$) and this was still true when adjusted for age and smoking ($P = 0.87$).
The mean blood flow responses (geometric means) to GTN ointment, placebo ointment and no treatment for the three groups are shown in Table 1 and illustrated in Fig. 2.

There was significantly increased blood flow response to placebo ointment compared with no treatment ($P < 0.001$) and to GTN compared with placebo ointment ($P = 0.004$).

The change in blood flow over time differed significantly between placebo and GTN ointment application ($P < 0.001$), but not between placebo and no ointment application ($P = 0.33$). It is to be noted that skin blood flow continued to increase at 10 and 20 min post-application of GTN ointment in all groups, whereas blood flow diminished over the same period of time after placebo or no ointment application in all groups.

There were no significant differences in responses between the three subject groups.

---

**Table 1.** Blood flow responses to no treatment, placebo ointment and topical GTN in control, PRP and LCSSc subjects. The results for each group are expressed as geometric means of flux in perfusion units (95% confidence intervals) at baseline (Base), and immediately (0 min), 10 and 20 min after ointment application.

<table>
<thead>
<tr>
<th>Group</th>
<th>No treatment</th>
<th>Placebo</th>
<th>GTN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base (0 min)</td>
<td>10 min</td>
<td>20 min</td>
</tr>
<tr>
<td>Control</td>
<td>142 (110, 184)</td>
<td>154 (128, 187)</td>
<td>148 (110, 184)</td>
</tr>
<tr>
<td>PRP</td>
<td>107 (84, 131)</td>
<td>129 (104, 168)</td>
<td>112 (95, 139)</td>
</tr>
<tr>
<td>LCSSc</td>
<td>140 (104, 180)</td>
<td>153 (126, 184)</td>
<td>145 (111, 180)</td>
</tr>
</tbody>
</table>

---

*Fig. 1.* Typical flux maps from a LCSSc patient (a) before and (b) 20 min after application of GTN/placebo. Index finger, GTN. Middle finger, petroleum jelly. Ring finger, no treatment.
Discussion

We have shown that topically applied GTN results in digital vasodilation in doses which are insufficient to produce any significant systemic effect, as evidenced by the lack of vasodilation in the ‘non-treated’ finger. The vasodilation observed in response to GTN was similar in all three subject groups. Admittedly, rubbing in placebo ointment also caused a vasodilatory response, but this was significantly less than with GTN, and was shorter lived. Our findings are consistent with those of Tucker et al., who recently reported that microvascular flow increased in response to topical application of a nitric oxide-generating gel in patients with PRP as well as in healthy control subjects. However, patients with SSc were not studied. Khan et al. also reported increased skin perfusion in healthy controls in response to a locally applied nitric oxide donor (S-nitrosothio-acetylglucose).

These findings have two main implications. First, with respect to the pathophysiology of SSc, nitric oxide donation via ointment application was effective at producing vasodilation even though several of the patients had well-established disease and one had severe and six had moderate skin involvement. While we have already demonstrated that dermal microvessels respond to iontophoresed nitroprusside, even in patients with very thickened skin, we were not sure whether the GTN would diffuse easily through thickened skin. The exact role of nitric oxide in the pathogenesis of SSc is incompletely understood. Although expression of inducible nitric oxide synthase has been found to be increased in sclerodermatous skin and there is some evidence for increased systemic circulating levels of nitric oxide in patients with SSc, there are persuasive arguments in favour of nitric oxide deficiency in patients with SSc: nitric oxide deficiency leads to vasoconstriction and a proliferative response of vascular smooth muscle, both of which are characteristic features of SSc. Our results lend further support to the suggestion that whatever the pathogenesis of the neuroendothelial dysregulation which is thought to occur in SSc, this can be over-ridden, at least in part, by exogenously supplied nitric oxide.

Second, topical GTN might be an effective treatment in patients unable to tolerate systemic vasodilators or unwilling to take these. Admittedly, the preparation was greasy, but nonetheless a proportion of patients might find this an acceptable form of treatment, especially SSc patients with prolonged ischaemia of a single digit. None of the patients or healthy control subjects experienced adverse effects. In this study we only examined acute effects as measured by laser Doppler imaging and did not study effectiveness over time. Therefore, further studies are required to examine this further.

Another observation from this study is the enormous potential of the new technique of laser Doppler imaging (scanning laser Doppler) to quantify treatment responsiveness objectively in patients with Raynaud’s phenomenon. Unlike conventional infrared thermography, laser Doppler gives a direct measure of dermal microvascular flow. Previous studies using laser Doppler in patients with Raynaud’s phenomenon have usually used the well-known single probe technique, which brings with it the inherent problem of site-to-site variability. The advantages of the newer technique of laser Doppler imaging are that it measures blood flow over an area rather than at a single site and that it is non-contact. It is likely that in the near future the technique of laser Doppler imaging will be widely applied in studies of digital blood flow in patients with primary and secondary Raynaud’s phenomenon, and in studies of other diseases affecting the microvasculature.

In conclusion, digital blood vessels respond to topical GTN not only in patients with PRP but also in patients with SSc, even in patients with very thickened skin. This is in the absence of significant systemic effects, as evidenced by the absence of adverse effects and by the lack of a rise in microcirculatory flow in the ‘untreated’ finger. GTN ointment therefore warrants further study in patients with both primary and secondary Raynaud’s phenomenon.
Acknowledgements

Dr Marina E. Anderson is an Arthritis Research Campaign-funded Clinical Research Fellow. The laser Doppler imager was funded by a Joint Research Equipment Initiative grant (contributions from the Medical Research Council, the Scleroderma Society and Moor Instruments Ltd).

References