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Reduced perfusion in systemic sclerosis digital ulcers (both fingertip and extensor) can be increased by topical application of glyceryl trinitrate☆

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ABSTRACT

Objectives: In patients with systemic sclerosis (SSc), fingertip digital ulcers (DUs) are believed to be ischaemic, and extensor surface DUs a result of mechanical factors/microtrauma. Our aim was to assess blood flow response to topical glyceryl trinitrate (GTN) compared to placebo in SSc DUs, looking for differences in pathophysiology between fingertip and extensor lesions.

Method: This was a double-blind, randomised, crossover, placebo-controlled study. Sixteen (6 fingertip, 10 extensor) DUs were each studied twice (one day apart): once with GTN and once with placebo ointment. Perfusion at the DU centre ('DUCore') and periphery ('DUPeriphery'), as measured by laser Doppler imaging was performed before and immediately after ointment application, then every 10 min, up to 90 min post-application. We calculated the area under the response curve (AUC) and the ratio of peak perfusion to baseline, then compared these between GTN and placebo.

Results: Perfusion was lower in the DUCore compared to the DUPeriphery (ratio of 0.52). The microvessels of the DUCore were responsive to GTN, with an increase in perfusion, with a similar effect in both fingertip and extensor DUs. The AUC and peak/baseline perfusion difference in means (ratio, 95% confidence interval) between GTN and placebo at the DUCore were 1.2 (1.0–1.6) and 1.2 (1.0–1.5) respectively, and at the DUPeriphery were 1.1 (0.8–1.6) and 1.0 (0.9–1.2) respectively.

Conclusion: DUs (both fingertip and extensor) were responsive to topical GTN, with an increase in perfusion to the ischaemic DU centre. If both fingertip and extensor DUs have a (potentially reversible) ischaemic aetiology, this has important treatment implications.

1. Introduction

Digital ulcers (DUs) are a major cause of pain and disability in patients with systemic sclerosis (SSc) (Bérezné et al., 2011; Mouthon et al., 2014), and are a biomarker of disease progression, including death (Mihai et al., 2016). However, relatively little is known about SSc-related DU pathophysiology. It is currently believed that fingertip DUs are ischaemia-driven, while those which occur over the extensor surfaces are related to mechanical abnormalities and microtrauma (Hachulla et al., 2007). Previous studies using laser-based techniques are supportive of an ischaemic component to DUs, with reduced perfusion to the centre of the DU compared to the periphery (Ruaro et al., 2015), including in extensor surface DUs (Murray et al., 2016). In addition, the tissue adjacent to DUs has been reported to be relatively hyperaemic compared to normal skin (Murray et al., 2016).

Whether DUs are ischaemic is a key clinical question because drug therapies, in general, rely upon vasodilation, to increase perfusion to the DU. A number of recent randomised controlled trials have excluded extensor DUs (Matucci-Cerinic et al., 2011; Hachulla et al., 2016; Khanna et al., 2016), presumably on the basis that if these ulcers are not ischaemic, then they are unlikely to benefit from vasoactive therapies.

Supplementation of the nitric oxide (NO) pathway is an important therapeutic strategy in the management of digital vascular disease in SSc (e.g. with phosphodiesterase type 5 inhibitors). In addition, we have previously reported that topical glyceryl trinitrate (GTN) (a NO

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donor) increases blood flow in *intact* SSc skin (Anderson et al., 2002) as measured by laser Doppler imaging (LDI).

Against this background, our aim was to assess the responsiveness of the microvessels in the centre ('DU Core') and adjacent tissue ('DU Periphery') of SSc-related DUs to topical NO donation from GTN, compared to placebo ointment, and whether this differs between fingertip and extensor DUs, to better inform our understanding of the pathogenesis (with therapeutic implications) of DUs in SSc. Our rationale was that if DU blood flow increases with GTN, then this would imply that the microvessels within DUs are capable of endothelial-independent vasodilation and that topical NO donation might be an effective therapy.

2. Patients and methods

2.1. Patients

Sixteen patients (13 female and 3 male) with SSc (7 lcSSc and 9 dcSSc) (LeRoy et al., 1988), with 6 fingertip and 10 extensor ulcers were studied. The mean (SD) age of patients was 55.7 (17.3) years. Raynaud's phenomenon (RP) duration (mean, SD) was 16.7 (8.8) years and disease duration (from first non-RP clinical manifestation) was 14.5 (8.6) years. All the patients had a history of previous DUs, often requiring previous prostanoid infusions (n = 11) and not uncommonly surgical debridement (n = 8) for severe digital vascular disease. SSc-associated autoantibodies were present in most patients, namely anticentromere (n = 3), anti-Scl-70 (n = 7) and anti-RNA polymerase III (n = 2). The majority of patients were receiving current vasodilatory treatment/s (n = 14), most commonly with calcium-channel blockers (n = 10), and with several patients receiving treatment with an endothelial receptor antagonist (n = 3) or PDE5-inhibitior (n = 1). The study was approved by the National Research Ethics Committee – Preston and all patients provided signed informed consent.

We did not use a particular DU definition in the study. Previous clinical trials have used different DU definitions: we adopted a pragmatic, 'real-world' approach. Two clinicians (MH and AH) with an interest in SSc-related DUs assessed the lesions prior to recruitment into the study. Salford Royal NHS Foundation Trust is a tertiary referral centre for SSc (including participation in previous DU clinical trials), and many of the patients included in the study had a history of severe digital vascular disease. Therefore, taken together, it is likely that the patients in our study were representative of those who would be included in SSc-related clinical trials. Patients with eschar overlying the DU were included, if the clinician felt the thickness was only minimal, and unlikely to have any detrimental effect on the potential impact of the ointments.

2.2. Study protocol

Patients were randomised (with a balanced allocation within DU subgroup - fingertip versus extensor) to receive either GTN or placebo ointment on day one, and the alternative the following day. Sealed envelopes contained the patients' allocation schedule, allowing the medication to be dispensed. Patients, and the two operators, one of whom applied the study medication and the second of whom performed the LDI (and later extracted LDI data), were all blinded to the randomisation. This approach with two operators minimised any bias from any potential information gained from applying the ointment (e.g. patient opinion, including any side effects). One patient received intravenous iloprost on day one after the first study visit; however, the second visit was over 12 h after completion of the infusion.

Patients were advised to refrain from caffeine containing beverages and smoking for a period of at least 4 h before each study visit. No changes were made to patients' existing vasodilatory therapy. After a 20 minute period of acclimatisation at 23 °C, baseline LDI of the DU was performed, using a modified MoorLDI-vr (Moor Instruments, Axminster, United Kingdom) LDI (red, 633 nm). Immediately after the initial image (or 'flux map') was acquired, either 200 mg GTN (2% Percutol ®) or placebo ointment (of similar appearance and consistency to GTN preparation) was applied to the DU, using a sterile applicator, and with a circular motion, for 1 min. Any visible excess ointment was promptly removed using gauze. LDI was performed immediately (time 0) after application of the ointment, and then every 10 min, up to 90 min, at each study visit. Imaging was terminated if the patient indicated a desire to stop. Patients were asked to report any side effects experienced during the study visits.

2.3. Image analysis

Perfusion data were extracted from the captured images. Using the LDI grey-scale image of the DU (to avoid bias from seeing the perfusion image), regions of interest (Fig. 1) of the same size were created to extract perfusion data from the DUCore and the DUPeriphery for each treatment visit. We chose to examine the DU adjacent skin because of the relative hyperaemia (as previously described), which could be important in DU healing.

2.4. Statistical analysis

Summary measures of each patient's response to GTN and placebo: area under the curve (AUC) and ratio of peak perfusion compared to baseline were calculated. We calculated the difference in means and a 95% confidence interval (95% CI) for each of these summary measures, accounting for the correlation between paired measurements. For both measures we calculated the difference in means and 95% CI on a logarithmic scale due to distributional skewness, before back-transforming to the original scale. This yielded values representing the ratio of GTN response compared to placebo. For two patients who did not complete the full observation period, we calculated AUC and peak/baseline perfusion restricted to the same amount of time in the comparator period, to ensure a like-for-like comparison. All statistical analyses were performed using STATA version 13.

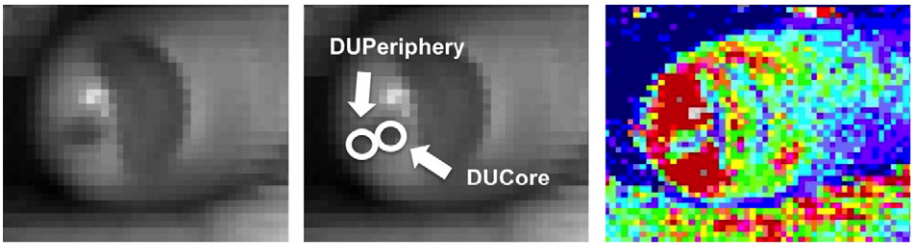

Fig. 1. LDI regions of interest. Illustration of how the regions of interest (ROI) were extracted to measure DU perfusion. Left and middle: identical grey scale images of a fingertip DU, the middle image illustrate the ROI of the DUCore and DUPeriphery. Right: The corresponding LDI perfusion (flux map) image of the DU. Blue indicates low perfusion, whereas, red is relatively higher perfusion. The perfusion to the DUCore is lower (i.e. ischaemic) compared to the DUPeriphery.