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Original article

Characterising the time-course of microvascular vasodilator responses in humans using laser doppler fluximetry and iontophoresis

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ABSTRACT

Introduction: Laser Doppler Fluximetry (LDF) and iontophoresis of vasodilators have been combined to assess microvascular flow and vasodilator reactivity in humans for many years and their use established. However, traditional data analysis methods measure the magnitude of flux change but not its time-course, a factor that may be of importance in defining vascular health. The aim of this study was to develop and utilise a novel data analysis method using a standardised LDF and iontophoresis protocol, assessing time to peak vasodilation (Tmax). **Methods:** Endothelial-dependent and -independent vasodilator responses (using acetylcholine and sodium nitroprusside respectively) were measured in the perimalleolar region both supine and standing in patients with isolated superficial venous insufficiency (ISVI) ($n=24$) and controls ($n=20$), on two occasions. Tmax was measured at each post-iontophoretic period. **Results:** Tmax was slower while standing and in patients with ISVI. There was no statistical difference in Tmax between visits for both groups ($p>0.05$), with coefficients of variation being between 20.2%–25.4%. ISVI, position and vasodilatory agents were significant determinants of Tmax. **Discussion:** Tmax appears to be a reproducible method of assessing venous vasodilation, is not affected by the dose and is impaired in patients with of ISVI in both the supine and standing positions.

1. Introduction

LDF and iontophoresis of vasodilators have been combined to assess microvascular flow and vasodilator reactivity for many years and their use has been established (Guy, 1998; Lenasi & Strucl, 2008). However, the reliability and repeatability of parameters measured from the test data, e.g. whether mean perfusion or maximum perfusion or area under the curve should be used to measure vasodilator responses, is unclear. Traditional data analysis methods measure the magnitude of the change in flux but not the time-course of that change, a factor that may be important in defining vascular health and consequently arterial tone, blood pressure and blood flow. An alternative approach would be to analyse the data to take account of the time taken to reach maximum perfusion (i.e. Tmax), or a specific component of the response.

Tmax (time-to-peak perfusion) has been measured during periods of reactive hyperaemia (Ninet & Fronek, 1985; Ray, Buckenham, Belli, Taylor, & Dormandy, 1997) with intra and inter-day coefficients of variation of <10% (Yvonne-Tee, Rasool, Halim, & Rahman, 2005). The speed and magnitude of vasodilation linked to the concept of "microvascular stiffness" are potentially of clinical importance perhaps as an index of vascular resistance (Wahlberg, Line, Olofsson, & Swedenborg, 1994) and may be a useful measure of microvascular function, especially in specific patient groups (e.g. ISVI or diabetes) where the combination of lower and slower endothelial vasodilation might imply a greater risk of tissue damage.

Therefore, the aims of this study were to measure Tmax at each stage of a standardised LDF and iontophoresis protocol, determine the reproducibility of Tmax and explore clinically important factors that may affect Tmax including a clinical condition (e.g. ISVI).

2. Materials and methods

Patients with uncomplicated ISVI, i.e. long or short saphenous vein reflux confirmed by duplex scanning, were recruited from referrals to the local Vascular Laboratory. Patients with present or past venous ulceration, arterial disease or major skin changes (including tissue oedema) in the gaiter area were excluded. Healthy controls were recruited from the research database in the Division of Vascular Medicine, and the absence of ISVI confirmed by duplex scanning. This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and all participants gave written informed consent. The protocol was approved by the Southern Derbyshire Local Research Ethics

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committee. Sample size calculations were based on our previous work on similar study groups for a significance level of 5%, and 85% power (Klonizakis et al., 2003).

Following baseline screening, including measurement of height (metres), weight (kilograms), high density lipoprotein, total cholesterol and blood pressure (BP mm Hg), participants attended the Clinical Research Unit on two separate study mornings 2 weeks (±2 days) apart. Microvascular perfusion and vasodilator responses in the gaiter area were measured in a temperature-controlled room according to a standardised protocol on both study days. Measurements were undertaken in the morning hours (8:30–10:30), with participants asked to refrain from smoking and ingesting any food, tea and coffee intake prior to the experiment.

2.1. Study periods

Following 30 min rest with the leg supported at a 30° angle above the heart level, the gaiter area was cleaned with alcohol and dried. Two Perspex iontophoresis chambers were positioned over healthy skin (avoiding any area of lipodermatosclerosis or superficial veins), approximately 2–5 cm apart on the surface of the leg 4–8 cm proximal to the medial malleolus. 1% acetylcholine (ACh) (Sigma Chemicals, UK) and 1% sodium nitroprusside (SNP) (Nipride, Roche Pharmaceuticals Ltd) diluted in deionised HPLC-grade water was injected into the anodal and cathodal iontophoresis chambers respectively. Drug concentration and current were chosen as such in order for non-specific vasodilatory effects to be minimised (Droog et al., 2004). The laser Doppler probe was positioned through the centre of each chamber.

LDF measurements were made using the DRT4 (Moor Instruments, Axminster, UK) included skin temperature, flux and microvascular dose–response curves for each of the four iontophoretic challenges obtained.

After achieving a stable recording of baseline flux, LDF responses to Ach and SNP were measured using an incremental-dose iontophoresis protocol, as previously described (Klonizakis et al., 2006). In brief, dose–response curves for ACh and SNP induced vasodilation were characterised using the following procedure to apply incremental charge-stimuli: 25 μA applied for 10 s (i.e.250 μCb), 50 μA for 10 s (500 μCb), 100 μA for 10 s (1000 μCb), and 100 μA for 20 s (2000 μCb), with a 4 min recording period between each dose. Thereafter, following a 10 minute recovery period, the chambers were repositioned at exactly the same site and baseline flux recordings were stabilised in the upright position, ensuring that baseline perfusion measurements returned to baseline prior to the 2nd part of the test. Measurements of vasodilator responses were repeated with the subject upright. The protocol was repeated on Visit 2.

2.2. Data analysis

Tmax (seconds), defined as the time taken for each individual subject to reach their maximum perfusion (measured in Perfusion Units [PU]) in response to the incremental-dose was measured for each 4-minute post-dose period with Tmax being measured from the end of each administrated dose. Graphical illustration of process for ACh for a single participant is presented in Fig. 1.

Tmax was measured twice on separate days by a single investigator (MK) for each recording. Values were extracted from each graph and verified by the raw dataset for the duration of the protocol. Reproducibility was determined by comparison of the results from Visit 1 and 2, for all the applied charges, both agents (ACh and SNP) and both positions (supine and standing).

2.3. Statistical analysis

Data obtained using a commercial software system (Moorsoft V1, Moor Instruments, UK) was transferred to a database (Excel 97) and analysed using statistical software (SPSS version 12, SPSS, USA). Coefficients of Variation (CV) were used to determine the reproducibility of the technique in the supine and standing positions. Mann Whitney *U*-Test and Bonferroni corrections were used where appropriate. Shapiro–Wilks Tests assessed the degree of normality for all baseline and outcome variables. Data is presented as mean (SD), with effect sizes (η^2) being measured using Cohen's *d* or Cramer's φ, depending on the variable type. Correlation and equivalency tests (in the form of Pearson's product-moment coefficient and Fisher's exact tests) were used to determine the relationship between a number of methodological (position, vasodilatory agent, and dose), clinical and demographic factors (presence or absence of ISVI, gender, age, smoking habits, exercise and alcohol consumption). Based on the results of these tests an ANCOVA model (including ISVI, position and the use of vasodilatory agents) was developed to determine their influence on Tmax.

3. Results

Forty four participants (20 controls and 24 ISVI), age and gender matched completed the study. The mean age of the participants was 50 (15) years, while 23 (52%) female and 6(14%) were current smokers. Separate demographics for each group are shown in Table 1. All participants were included in the analysis described in this report.

3.1. Tmax reproducibility

There were no significant differences in Tmax between visits ($p>0.05$) (averaged values for each position and agent presented in Table 2, separately for each group).

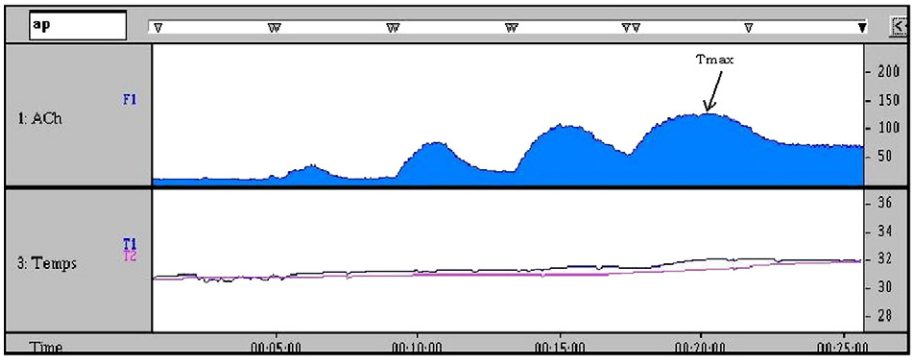

Fig. 1. Graphical depiction of Tmax measurement.