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Laser Doppler imaging in the detection of peripheral neuropathy ☆

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ABSTRACT

Small fiber neuropathy is common in a number of systemic diseases and is often challenging to diagnose. Laser Doppler imaging (LDI) is a test of small fiber neurovascular function that can quantify the integrity of the vasomotor C-fiber mediated axon-reflex, but no standardized method of analysis exists. We developed a novel LDI analysis technique and tested it in a human model of small fiber neuropathy. Eighteen healthy subjects (age 24 ± 3 years) underwent LDI testing to assess the axon-mediated flare area in response to 10% acetylcholine iontophoresis. LDI measurements were taken before and longitudinally after a 48-hour application of 0.1% capsaicin (to cause a transient small fiber neuropathy) on the skin of the thigh; placebo cream was placed on the contralateral thigh as a control. We compared our new LDI image analysis technique to two previously published methods. The new LDI analysis technique was the only method to show a consistent difference in axon-reflex area between capsaicin treated and placebo treated skin on all testing days ($p < 0.05$) with maximum attenuation of the flare area immediately post-application (438 ± 298 mm^2 vs. 824 ± 375 mm^2, $p < 0.05$). In conclusion, this study demonstrates that our novel flare area method for LDI analysis can detect neurovascular dysfunction in a model of small fiber neuropathy, is an improvement over existing methods, and may supplement clinical assessment of small fiber neuropathy.

1. Introduction

Peripheral small fiber neuropathy is present in a number of diseases including amyloidosis, paraneoplastic syndromes, diabetes and other glucose dysregulated states (Freeman, 2005). Neuropathy may variably involve both somatic and autonomic small nerve fibers (Tavee and Zhou, 2009; Gibbons et al., 2010a). The diagnosis of small fiber neuropathy is often challenging because standard electrodiagnostic tests, which primarily assess large fiber function, are frequently normal in these patients (Tavee and Zhou, 2009). Laser Doppler analysis of cutaneous blood flow is a novel test of small nerve fiber function (Berghoff et al., 2006; Caselli et al., 2006). Techniques include the use of single-point laser-Doppler flowmetry (LDF) or 2-dimensional laser Doppler imaging (LDI) to investigate the integrity of the neurogenic axon-reflex when combined with iontophoresis of a cholinergic agonist (Krishnan and Rayman, 2004; Berghoff et al., 2006).

Over the past several decades, a number of studies confirm that LDI and LDF are able to detect differences in vasomotor function between groups of individuals with and without neuropathy (Benarroch and Low, 1991; Bickel et al., 2002; Krishnan and Rayman, 2004; Berghoff et al., 2006; Gibbons et al., 2010b). However, most investigators agree that LDF is not sensitive enough to detect neuropathy in individual subjects (Parkhouse and Le Quesne, 1988; Benarroch and Low, 1991; Caselli et al., 2006). In contrast, many investigators feel LDI is more reliable, but there are several proposed methods for LDI analysis and the optimal approach is not known (Bickel et al., 2002; Kramer et al., 2004; Green et al., 2009).

In this paper, we report a novel LDI analysis technique and compare the results against two previously published LDI analysis methods (Kramer et al., 2004; Green et al., 2009).

2. Methods

2.1. Study design

This was a randomized double-blind placebo controlled longitudinal study of LDI in eighteen healthy subjects. Subjects had 0.1% capsaicin cream applied in a 48-hour occlusive dressing to one anterior thigh to create a standardized, reversible small fiber neuropathy as previously described (Polydefkis et al., 2004; Gibbons et al., 2010b). Subjects had placebo cream applied to the contralateral thigh. LDI was measured before and weekly for 4 weeks after capsaicin/placebo application.

Baseline LDI measurements were collected on both anterior thighs of each subject on day 1. After LDI testing, subjects had a 48-hour application (days 1 and 2) of capsaicin cream (2.4 g of 0.1%, Chattem

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Inc., Chattanooga, USA) to one anterior thigh and placebo cream (Johnson & Johnson, New Brunswick, NJ, USA) to the contralateral thigh in occlusive dressings over a 50 × 80 mm^2 area. Capsaicin and placebo creams were randomly assigned to the right or left anterior thigh using block randomization. LDI measurements were repeated on both thighs on days 3, 10, 17, 24 and 31. Three-millimeter punch skin biopsies were taken on day 17 in both capsaicin and placebo treated regions to measure intra-epidermal nerve fiber density.

2.2. Subjects

Eighteen healthy subjects (10 females, 8 males) ages 21 to 27 years (mean: 24 years) were enrolled. None of the subjects had evidence by history or exam of neuropathy, peripheral vascular disease, tobacco use, current medication use or other medical disease. The protocol was approved by the Beth Israel Deaconess Medical Center Institutional Review Board. Each subject signed a written informed consent.

2.3. Iontophoresis

Vasomotor axon-reflex mediated flare response was provoked through iontophoresis of 10% acetylcholine (Penta International Corporation, Fairfield, NJ, USA) at 0.4 mA for 140 s (Phoresor-PM850, IOMED, USA) using a drug delivery electrode with an internal diameter of 1.1 cm (LI 611, Perimed, Järfälla, Sweden) centered over the LDI scanning area as previously reported (Gibbons et al., 2010b).

2.4. Laser Doppler imaging (LDI)

Blood flow measurements were performed in the capsaicin and placebo exposed area of subjects placed in a semi-recumbent position with standardized lighting in a temperature controlled room (25 ± 1 °C) at day 1 and on days 3, 10, 17, 24 and 31. The subjects' legs were stabilized and immobilized through use of foam blocks. After a 20-minute acclimatization period, a laser Doppler perfusion imager (Periscan PIM III, Perimed, Sweden) using a stable helium neon gas laser (λ = 632.8 nm) measured cutaneous blood flow over a 4.2 cm × 4.4 cm area at a distance of 30 cm from the skin surface. Images were recorded in repeated image mode (36 pixels ×37 pixels (18.5 cm^2 image size), 1 mm step length, 28 pixels per second, 48 s per image). Five baseline images were obtained to quantify resting blood flow, followed by iontophoresis with acetylcholine to provoke an axon-reflex flare response and an additional 31 laser Doppler images were obtained.

2.5. Blood flow analysis

The laser Doppler imager records every image as a map of single point data cells with specific perfusion values. The values in each cell are reported in perfusion units (arbitrary blood flow values), but do not change across any analysis method. A perfusion 'flare' response is reported when the value of a cell exceeds a predetermined threshold. The selection of the appropriate threshold is what distinguishes each analysis method.

2.5.1. Analysis method 1: flare area method

Our new method to quantify the axon-reflex area determined the optimal perfusion threshold by measuring the maximum axon-reflex flare in all 18 subjects on day 1. The raw data from the images were exported into an excel file. The 5 baseline images for all subjects from day 1 were averaged and the mean blood flow across all cells is determined to be 111 PUs. We then established a cutoff threshold and the flare area was defined as the number of cells above the threshold after iontophoresis subtracted from the number of cells

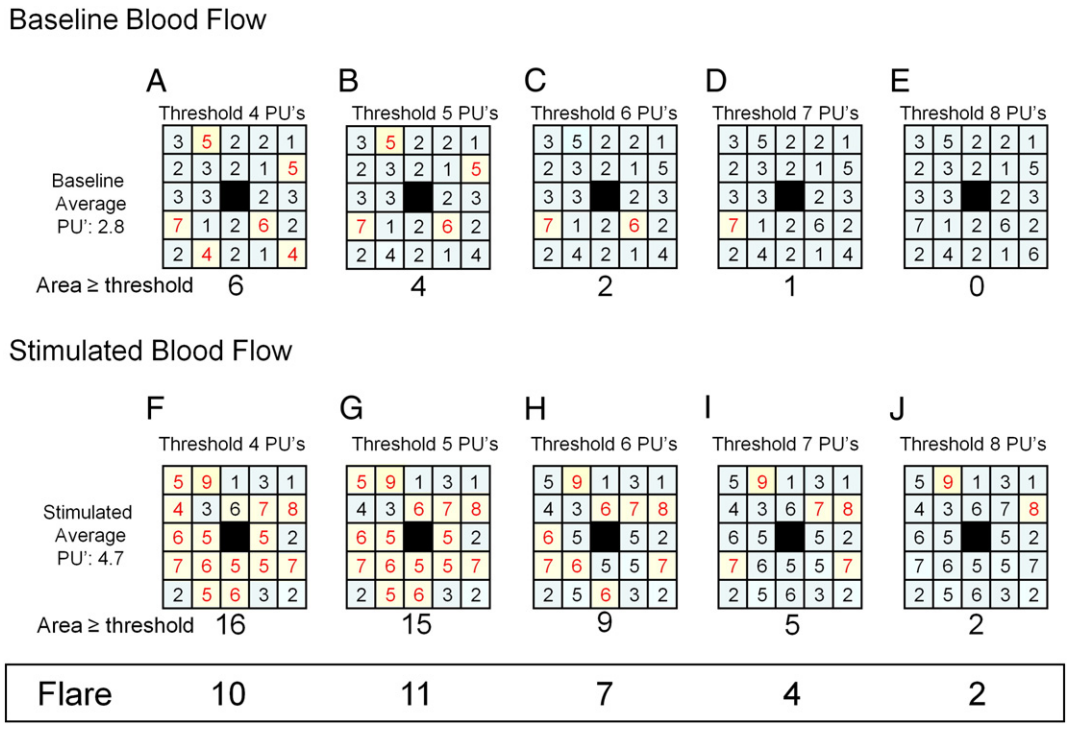

Fig. 1. Flare area method. This is an example of the flare area method. The top row of squares (A–E) shows the same baseline perfusion units (PUs) for a sample series of scanned images (this example shows a 5 × 5 grid for simplicity, the actual image would be 36 × 37 pixels). The center box of each square (shown in black) is the region of iontophoresis and is not included in the analysis. The values in each square that are ≥ to the perfusion threshold (listed at the top of each square — the threshold is 4 PUs in A, etc.) are highlighted in yellow and shown in red text. Thus, in square A, six boxes have values ≥4 PUs and are highlighted. The total number of highlighted boxes are counted and reported below each box (in A, the area exceeding the perfusion threshold is 6). The bottom row of squares (F–J) is the perfusion values post-iontophoresis. The data values are tested against a series of increasing thresholds ranging (in this example) from 4 to 8 (A–E and F–J). The difference in flare area between the baseline (A–E) and stimulated (F–J) region is listed at the bottom of the figure in the box entitled "Flare". In this example, the largest Flare response is with the threshold set to 5 PUs where baseline flare area of 4 (B) and the stimulated flare area of 15 (G). The difference between baseline and stimulated areas is a flare area of 11.