Determination of Capsaicin Induced Increase in Dermal Blood Flow Using Laser Doppler Flowmetry Technique

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Received April 28th, 2011; revised May 20th, 2011; accepted June 30th, 2011.

ABSTRACT

In the present study, we evaluated laser Doppler flowmetry technique using LDF100C (Biopac systems) by determining blood flow changes following acute application of capsaicin on 12 healthy human subjects. Capsaicin applied locally (topical application) at a dose of 0.075% produced significant increase in mean dermal blood flow from 31.4 ± 3.1 Blood Perfusion Units (BPU) to 115.7 ± 24.6 Blood Perfusion Units (BPU) after 30 minutes, also there was significant difference in dermal blood flow change between placebo (32.1 ± 2.7 BPU) and capsaicin (115.7 ± 24.6 BPU) application. Capsaicin application produced significant percentage change in dermal blood flow by 291.0 ± 85.3% from baseline, while the change was insignificant with placebo (13.2 ± 7.4%). Therefore, it is suggested that this technique which is technically sound, non-invasive and inexpensive can be adopted in various fields of research to determine blood flow changes and this technique can also be utilized to determine the antagonists of the mediators involved in capsaicin induced vasodilatation.

Keywords: Pharmacodynamics, Neurogenic Inflammation, Capsaicin

1. Introduction

Laser Doppler Flowmetry (LDF) is an established and reliable method for measurement of blood perfusion in microvascular research. Laser Doppler signals from the tissue are recorded in BPU (Blood Perfusion Units) which is a relative units scale defined using a carefully controlled motility standard comprising a suspension of latex spheres undergoing Brownian motion. The LDF technique (Figure 1) offers substantial advantages over other methods in the measurement of microvascular blood perfusion. This technique provides promise and opportunity to adapt the methodology in various fields of research for example in cerebral monitoring (stroke, injury …), transplantation surgery (skin grafts, free flaps …), vital organ monitoring (organ viability), tumor vascular research (angiogenesis) and peripheral vascular research (diabetes). Studies have shown that it is both highly sensitive and responsive to local blood perfusion and is also versatile and easy to use for continuous monitoring [1,2].

Capsaicin, the pungent ingredient in a wide variety of hot peppers, has been used extensively in human pain models to induce experimental pain [3-6]. Application of capsaicin to the skin activates the transient receptor potential vanilloid type 1 receptor (TRPV1), producing neurogenic inflammation and vasodilation [7,9-12]. Most evidence indicates that the release of calcitonin gene-related peptide (CGRP) is a major initiator of this response [13,8]. Other putative bioactive mediators are substance P (SP), neurokinin (NK) A, somatostatin, nitric oxide (NO), histamine and prostaglandins, but their role in capsaicin-induced neurogenic inflammation in the normal human skin is not well established [14-18].

The aim of the study was to determine microvascular changes following acute application of capsaicin using laser Doppler flowmetry Technique.

2. Materials and Methods

Twelve healthy, male subjects between 24 and 32 years old were enrolled. Mean ±SD (range) for age, weight, height and Body Mass Index (BMI) were given in Table
Table 1. Showing demographic characteristics of study participants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>27.8 ± 3.9</td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>68.6 ± 7.7</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>170.7 ± 4.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 2.0</td>
</tr>
</tbody>
</table>

Values are Mean ±SD.

Figure 1. Shows laser Doppler flowmetry technique. Low power laser light is used to illuminate tissue using a fibre optic; The light is scattered by the static tissue structures and moving blood cells; The moving blood cell impart a Doppler Shift; An adjacent fibre detects light returned from the tissue; This light contains Doppler shifted and Unshifted light; The signal is processed to extract the signal related to the moving red blood cells.

1. All completed study per protocol. Exclusion criteria included: obesity (body mass index >30 kg/m²), under weight (body mass index <18.5 kg/m²), any medication with the potential to alter cardiovascular or thermo-regulatory control or response, allergies to hot peppers, and various dermatological conditions or diseases.

The study protocol was approved by the institutional human studies committee, Nizam’s Institute of Medical Sciences, Hyderabad, India and complied with the Declaration of Helsinki on Biomedical research Involving Human Subjects. Before enrolment, all participants gave informed consent in writing.

Each participant had an initial visit to the experimental laboratory, for a physical examination and a medical history assessment. A 0.075% capsaicin cream (Asian Herbex LTD, Hyderabad, India) was applied on the volar aspect of forearm of one hand to detect any adverse reactions to capsaicin. In the absence of symptoms indicating hypersensitivity, including an unusually painful or hyperemic response to capsaicin, the subjects were cleared to participate in the study.

A Laser Doppler Flowmetry amplifier (BIOPAC Systems, Goleta, CA, USA) with an associated Laser Doppler Flowmetry probe (TSD 142) was used to perform the necessary measurements. Experiment was performed in environmental conditions where the temperature was maintained at ~23°C ± 2°C.

Application site (10 cm distal to elbow crease) were marked on the volar aspect of the forearm of both non-dominant and dominant hand with a soft pen. Prior to any applications, Laser Doppler Flow (LDF) recording were taken on volar aspect of both the forearms to serve as a baseline measurement.

After the initial measurement, acute capsaicin applications (0.075%) were preceded on the non-dominant forearm and placebo (containing ointment base only) application to the dominant forearm. The blood flow response was measured at 30 minutes post-capsaicin/placebo application in the area within ring (Figure 2).

Statistical Analysis

Participant’s demographic data is presented as mean ± SD, and pharmacodynamic parameters are presented as mean, Standard Deviation (SD), Standard Error (SE) and 95% of Confidence Interval (CI).

To find out the differences between placebo and capsaicin treatment one way ANOVA was used. Paired t-test was used to analyse the difference within the group, and between the groups respectively. p < 0.05 was considered for statistical significance. All the statistical analysis...
were performed using the Graph pad PRISM software 4 (Graph pad software Inc. San Diego, California, USA).

3. Results

Dermal blood flow was determined in 12 healthy male subjects’ with mean age, weight, height, and BMI (27.8 ± 3.9 yrs), (68.6 ± 7.7 kgs), (170.7 ± 4.9 cms), and (23.5 ± 2.0 kg/m²) respectively shown in Table 1.

Application of capsaicin didn’t produce any major adverse events; however, there was local flare and mild tingling sensation which disappeared after 4 hrs of application.

There was no difference in blood flow at baseline between placebo and capsaicin forearm. Dermal blood flow significantly increased in all the 12 subjects’ thirty minutes after 0.075% application of capsaicin ointment. Dermal blood flow represented as Blood Perfusion Units (BPU) obtained with placebo and capsaicin is given in Table 2.

After 30 minutes of capsaicin application the mean dermal blood flow significantly increased from 31.4 ± 3.1 BPU to 115.7 ± 24.6 BPU, this increase was significant (p < 0.001). Similarly, there was a significant difference in dermal blood flow change after 30 minutes between placebo (32.1 ± 2.7 BPU) and capsaicin (115.7 ± 24.6 BPU) application (p < 0.001).

The percentage change from baseline in dermal blood flow in placebo arm and in capsaicin arm is shown in Figure 3. Placebo application produced insignificant rise in blood flow by 13.2 ± 7.4%, while there was a significant increase in dermal blood flow 30 minutes after the application of 0.075% capsaicin (291.0 ± 85.3%). This difference between placebo and capsaicin was highly significant (p < 0.001).

4. Discussion

This is the first study to determine capsaicin-induced neurogenic inflammation using Laser Doppler flowmetry technique.

In the present study we have demonstrated the effect of local application of capsaicin on microcirculation of forearm skin in healthy male subjects using laser Doppler flowmetry technique. Earlier technique of Laser Doppler perfusion imaging has been described to have good reproducibility to detect microvascular changes on skin surface.

Exploration of microcirculation by laser Doppler technology has often been considered poorly reproducible [19]. Introduction of laser Doppler perfusion imaging has considerably improved reproducibility of this technique [20-22]. As the clear guideline for measurement of digital blood flow by laser doppler perfusion imaging are available now, it has been regularly used to assess cutaneous blood flow of normal and irritated skin [20]. Earlier study has revealed a dose-dependent increase in digital blood flow after capsaicin application on forearm [23].

In the present study application of capsaicin (0.075%)
significantly increased dermal blood flow as compared to placebo. Van der Schuere et al., have demonstrated the increase in blood flow after application of 300 and 1000 micro grams of capsaicin while the application of 100 micro grams didn’t produce any increase in blood flow [23].

In our study the blood flow measurement was carried out after 30 minutes application of capsaicin. It has been shown that 1000 micrograms of capsaicin produced the maximum response between 30 and 45 minutes after application and at 60 minutes time point there was decrease in blood flow [23]. There was increase in blood flow (291 ± 85.3 %) with 0.075% capsaicin application compared to placebo in our study. Helme and McKernan have extensively investigated the wide variation in size and intensity of capsaicin induced flare response [7]. According to these authors, site of application and age were the factors responsible for large variation. Gazzerani et al. recently found that capsaicin induced sensory and vasomotor responses were also gender specific [24]. The present study was conducted only in male gender. Heterogeneity in the density and function of capsaicin sensitive nociceptive nerve endings of the dermal microcirculation seem to be the most plausible explanation. There may be variation in the response due to difference between the proximal and distal forearm, due to difference in skin thickness [23]. We have thus applied the capsaicin 10 cms distal to elbow crease to avoid this site variation.

Munce and Kenney reported decrease in local skin blood flow response in older participants than healthy males [25]. Age had a significant effect on local vasodilation with younger individuals having percentage of maximal cutaneous vascular conductance (% CVCmax) at least 2 times greater than seen in older group. Previous observations of decrease flare size in older individuals after acute application of capsaicin suggest that there is reduction in reduced skin blood flow in these subjects [7]. We included only younger individuals, the average age of participants in our study was 27.8 ± 3.9 yrs. Acute capsaicin stimulates a specific population of sensory nerves in the skin that possess capsaicin receptors, eliciting local release of vasoactive neurotransmitters from their nerve endings [25]. The further use of our model can have advantage in dose finding and proof of concept studies.

5. Conclusions

The present study has shown an increase in the dermal blood flow induced by topical application capsaicin on the human forearm using laser Doppler flowmetry technique. Hence, this pharmacodynamic model which is non-invasive, technically uncomplicated and sound, might therefore facilitate the early evaluation of antagonists of mediators involved in neurogenic inflammation, including CGRP, TRPV1 and possibly, SP antagonists in humans.

6. Acknowledgements

The study was funded through the Indian Council of Medical Research (ICMR) fund, Government of India. The authors declare no financial conflict of interest connected to this study and its results.

We thank the Director, Nizam’s institute for providing us the necessary infrastructure.

7. References


