Maximization of skin capillaries during intravital video-microscopy in essential hypertension: comparison between venous congestion, reactive hyperaemia and core heat load tests

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ABSTRACT

Intravital capillary video-microscopy is a dynamic method for studying skin capillaries. The technique of direct intravital microscopy (without dyes) depends on the presence of red blood cells inside capillaries for their identification. The aim of the present study was to compare different techniques to try to establish the best method for maximizing the number of visible perfused capillaries during intravital capillary microscopy. We compared the effects of venous congestion with those of post-occlusive reactive hyperaemia (Study 1). We also investigated venous congestion followed first by post-occlusive reactive hyperaemia and then by a core heat load test (Study 2). Finally we investigated venous congestion followed by post-occlusive reactive hyperaemia combined with venous congestion (Study 3). In Study 1, capillary density increased with venous congestion from a baseline value of 74 ± 2 (mean ± S.E.M.) per field to 82 ± 3 per field (P < 0.001); analysis of variance. With reactive hyperaemia, there was an apparent decrease in visible capillary density to 69 ± 2 per field. In Study 2, baseline capillary density was 69 ± 4 per field, and this increased significantly with venous congestion to 74 ± 4 per field (P = 0.01). With both reactive hyperaemia and core heat load, the apparent density was 62 ± 4 per field. In Study 3 the baseline density was 70 ± 3 per field, and this increased significantly with venous congestion to 80 ± 3 per field (P < 0.0001). With reactive hyperaemia combined with venous congestion, the density was 81 ± 3 per field (P = 0.328 compared with venous congestion alone). The results show that venous congestion at 60 mmHg for 2 min is the most effective method for visualization of the maximal number of perfused skin capillaries during intravital video-microscopy.

INTRODUCTION

Intravital capillary video-microscopy has allowed the dynamic study of skin capillaries in many diseases, including hypertension, diabetes mellitus, arterial occlusive disease and skin disorders [1–3]. In essential hypertension, many abnormalities are known to occur in the capillary circulation. These include capillary hypertension, increased looping, increased transcapillary filtration and a reduction in capillary density per volume of tissue [4–7]. Decreased capillary density, or rarefaction, is a consistent finding in patients with essential hypertension [7–11]. We have shown recently that much of the reduction in capillary density in patients with
essential hypertension is due to the structural (anatomical) absence of capillaries, rather than to functional non-perfusion [7]. In that study [7] we found that maximization of visible skin capillaries during intravital video-microscopy was much better with venous congestion than with post-occlusive reactive hyperaemia. This enhancing effect of venous congestion on the visualization of skin capillaries has been reported previously [12,13]. As the usual technique of direct intravital microscopy (without dyes) depends on the presence of red blood cells inside capillaries for their identification, this method therefore has the potential for missing non-perfused capillaries. It is assumed that capillaries, especially in the skin, work on a ‘rota system’, i.e. some are perfused while others are shut down. This would seem likely for the skin, where the blood supply far exceeds the nutrient requirements of the tissue [14]. As blood flow is controlled at the arteriolar level (pre-capillary resistance vessels), the hypothesis would be, that by reducing pre-capillary resistance through reactive hyperaemia [10] or core heat load [15], most or all of the capillaries would ‘open up’ and so maximize capillary numbers. On the other hand, venous congestion, by activating any veno-arteriolar response, may theoretically reduce flow to the capillaries and possibly decrease numbers of ‘functional’ capillaries. We noted [7] that, during the post-occlusive reactive hyperaemia test, there was an apparent decrease in the number of visible capillaries. It is well known that capillary blood cell velocity increases with post-occlusive reactive hyperaemia [16,17]. With reactive hyperaemia, the number of capillaries showing active flow motion increased [18]. Putting the last two findings together, it may not be surprising that less capillaries were visible during reactive hyperaemia than with venous congestion. Therefore combining reactive hyperaemia (to reduce pre-capillary resistance) with venous congestion (to increase red blood cell content inside capillaries) seemed to be a potentially better method for the visualization of maximal skin capillary density.

The aim of the present study was to compare different techniques in order to establish the best method for maximizing the number of visible perfused capillaries during intravital capillary microscopy. Visualization of the maximal capillary density will help to establish whether the observed rarefaction of skin capillaries in essential hypertension is due to the structural (anatomical) absence of capillaries, or to functional non-perfusion secondary to severe vasoconstriction upstream.

**METHODS**

**Subjects**
The study included patients with essential hypertension who had not received any previous treatment for their high blood pressure, and healthy normotensive volunteers. All subjects were assessed in the Blood Pressure Unit, St. George's Hospital Medical School. They were studied in the morning between 09.00 and 11.00 hours after an overnight fast. Patients with a history of connective tissue disease, diabetes mellitus or skin diseases, and those taking vasoactive drugs, were excluded from the study. The protocol was approved by the local Ethics Committee of St. George’s Hospital. Written informed consent was obtained from each patient.

The capillaroscopy studies were performed in a temperature-controlled laboratory (21–24 °C) after the study subjects had rested for at least 20 min while semi-supine. Subjects were seated with the left forearm and hand supported at heart level. Both the hand and the forearm were rested on a splint surrounded by a vacuum pillow (a specially constructed pillow filled with polyurethane foam that can be moulded to any desired shape by creating a vacuum) to immobilize the study site.

**Intravital capillaroscopy**
An epiluminated microscope with a 100 W mercury vapour lamp light source and a PL 6.3/0.2 objective (Wild-Leitz type 307-143.004; Leica UK Ltd), final magnification ×196, was used for video-microscopy. Microscopic images were recorded using a CCD (charge-coupled device) camera (Hitachi model CCD HV-725K), and were transferred using a video-scaler (VS-1000) and a video-timer (For-A VTG 33) on to a video recorder (Panasonic model AUC 7350) for storage. The skin of the dorsum of the middle phalanx of the non-dominant (left) hand was examined. Four microscopic fields (0.68 mm² each) centred around an ink spot were recorded continuously for 5 min so as to observe intermittently perfused capillaries. Still-frame video prints (Sony Multiscan Video-Printer UP-930) obtained from each recorded field were analysed off-line. The number of capillaries per field was counted by hand both from these prints and from live playback of the recorded tapes, in order to ensure flow within counted capillaries. Skin temperature was monitored throughout the study with a temperature probe (YSI Tele-thermometers) on the dorsum of the left index finger. Patients with cold hands or Raynaud’s phenomenon were excluded from the study.

**Study 1**
In this study, we compared venous congestion with post-occlusive reactive hyperaemia. A miniature blood pressure cuff was applied to the base of the left middle finger, and the cuff was then inflated and maintained at 60 mmHg for 2 min. Images were recorded using one of the four microscopic fields chosen at random. Venous congestion maximizes the number of capillaries visualized by increasing their red blood cell content. Reactive hyperaemia, on the other hand, produces a vasodilator...
Table 1  Baseline characteristics of study subjects
BMI, body mass index; NA, not applicable. Significance of differences: *P < 0.0001, **P = 0.012 compared with baseline density.

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>39</td>
<td>12</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>20/19</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.5 ± 2.6</td>
<td>39.8 ± 5.5</td>
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<tr>
<td>Weight (kg)</td>
<td>73.3 ± 2.5</td>
<td>75.3 ± 4.9</td>
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<tr>
<td>Height (cm)</td>
<td>171.7 ± 1.5</td>
<td>173.2 ± 2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 ± 0.8</td>
<td>25.2 ± 1.8</td>
</tr>
<tr>
<td>Supine blood pressure (mmHg)</td>
<td>135/82 ± 4/3</td>
<td>143/89 ± 11/8</td>
</tr>
<tr>
<td>Capillary density per field (0.68 mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74 ± 2</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>After 2 min of venous congestion</td>
<td>82 ± 3 *</td>
<td>74 ± 4 **</td>
</tr>
<tr>
<td>Post-occlusive reactive hyperaemia</td>
<td>69 ± 2</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>Core heat load</td>
<td>NA</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>Post-occlusive reactive hyperaemia combined with venous congestion</td>
<td>NA</td>
<td>NA</td>
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response mediated by myogenic and/or local chemical factors. At least 10 min after the release of venous congestion, arterial blood flow into the forearm and hand was stopped for 3 min by inflating a sphygmomanometer cuff applied to the upper arm at 200 mmHg. The cuff was then deflated abruptly by breaking the connection, and subsequent capillaroscopic images were obtained continuously for 2 min. This study included 39 subjects [20 males; age 42.5 ± 2.6 years (mean ± S.E.M.); mean supine blood pressure 135/82 ± 4/3 mmHg].

Study 2
In this study we investigated venous congestion followed by post-occlusive reactive hyperaemia, as described above, followed after a 10 min rest period by a core heat load test. This entails immersion of the legs of the subject, up to knee height, in a water bath maintained at 45 °C using a thermo-circulator (Harvard Instruments, Edenbridge, Kent, U.K.) for a period of 30 min [19]. This results in a reflex vasodilatation in the skin mediated by activation of sympathetic vasodilator fibres [15,20]. After each test, subjects had at least a 10 min rest period to allow the skin circulation to return to basal conditions. This study included 12 subjects (seven males; age 39.8 ± 5.5 years; blood pressure 143/89 ± 11/8 mmHg).

Study 3
In this study we investigated venous congestion followed by post-occlusive reactive hyperaemia combined with venous congestion. After occluding arterial blood flow in the forearm and hand for 3 min as above, the reactive hyperaemia response was measured for 30 s, then venous congestion was applied as described above. The number of perfused capillaries was counted throughout this procedure. This study included 30 subjects (19 males; age 43.6 ± 3.0 years; blood pressure 128/84 ± 7/4 mmHg).

Blood pressure
Blood pressure was measured with a semi-automatic oscillometric device (OMRON HEM705CP) with appropriate cuff size. Supine blood pressure was taken as the mean of three readings obtained at 1–2 min intervals. Body weight was recorded in the morning, after voiding, with the patients wearing indoor clothing and no shoes.

Statistical analysis
All results are given as means ± S.E.M. The data were processed using the package StatView 5.0 (SAS Institute Inc.). Analysis of variance (ANOVA) for repeated measurements and the Fisher post hoc test were used to compare groups.

RESULTS
Table 1 shows the characteristics of the study subjects, and capillary densities at baseline and after different techniques carried out to maximize perfused capillaries. In study 1, capillary density increased with venous congestion from a baseline value of 74 ± 2 per field (0.68 mm²) to 82 ± 3 per field (P < 0.0001; ANOVA). Reactive hyperaemia increased the number of capillaries with active red blood cell motion (functional density), but there was an apparent decrease in visible capillary density to 69 ± 2 per field (Figure 1). In Study 2 the
Capillary densities were measured at rest, with venous congestion (60 mmHg for 2 min) and with post-occlusive reactive hyperaemia (200 mmHg for 2 min). Horizontal black bars represent means.

Baseline capillary density was 69 ± 4 per field; with venous congestion this increased significantly to 74 ± 4 per field (P = 0.01; ANOVA). With both reactive hyperaemia and core heat load, the apparent density was 62 ± 4 per field (Figure 2). In Study 3 the baseline density was 70 ± 2 per field; this increased significantly with venous congestion to 80 ± 3 per field (P < 0.0001; ANOVA). When reactive hyperaemia was combined with venous congestion, the density was 81 ± 3 per field (P = 0.328 compared with venous congestion alone) (Figure 3).

**DISCUSSION**

The main finding of the present study is that venous congestion at 60 mmHg for 2 min allows visualization of a maximal number of skin capillaries during intravital video-microscopy. The results confirm our previous finding that the number of capillaries seen with venous congestion exceeds that seen with post-occlusive reactive hyperaemia [7]. We also confirm that the observed reduction in skin capillaries in essential hypertension is due to a structural abnormality rather than to functional non-perfusion [7]. This structural rarefaction of skin capillaries in essential hypertension seems to be due to a primary abnormality that antedates the onset of sustained hypertension, rather than being secondary to the elevation of blood pressure [21].

Why does venous congestion allow visualization of more capillaries than does reactive hyperaemia? During direct intravital microscopy without dyes, capillaries in the skin of the dorsum of the fingers are mostly seen with no or minimal active red blood cell motion. Only a few capillaries at a time are seen with highly active red blood cell motion. This indicates that most of the ‘closed’ or resting capillaries are usually filled with stagnant non-moving red blood cells, rather than being empty and non-visible. In other words, ‘closed’ capillaries on the dorsum...
of the fingers are easier to see by direct intravital microscopy, as they are filled with red blood cells, whereas active (open) capillaries are harder to see because of the relative paucity of red blood cells flowing through them. Venous congestion helps this by increasing the red blood cell content of capillaries, transforming those with highly active red blood cell motion into red-blood-cell-filled capillaries. Furthermore, capillaries with plasma-only perfusion become filled with red blood cells. The enhancing effect of venous congestion on the visualization of skin capillaries by video-microscopy has been reported previously [12,13,22].

On the other hand, post-occlusive reactive hyperaemia and core heat load each increase the number of capillaries with active red blood cell flow motion [18], or what is known as the functional capillary density [23]. It is well known that capillary blood cell velocity is increased by post-occlusive reactive hyperaemia [16,17]. It was therefore not surprising in our study that apparently fewer capillaries were visible during reactive hyperaemia than with venous congestion. The exact mechanism of reactive hyperaemia is not well understood, although it does not depend on vasomotor nerves [14]. One factor is thought to be the accumulation of vasodilator metabolites that are normally washed away or destroyed by circulating blood [24]. Another possible factor is a myogenic relaxation of vascular smooth muscle that may occur when the transmural pressure in the resistance vessels is reduced distal to the site of circulatory arrest [25]. Various durations of occlusion from 15 s up to 6 min were performed. For the study of post-occlusive reactive hyperaemia in finger capillaries, a 1-min arterial occlusion has been found in clinical studies to be optimal [16]. At high skin temperatures (> 30°C), the reactive hyperaemia response is limited because of local vasodilatation, and at about 34°C the response is almost completely absent [26]. The vasodilatory mechanism in core heat load, however, is different from the myogenic response following ischaemia in post-occlusive reactive hyperaemia. Body heating causes a large reflex vasodilatation in the hand, which is due to the release of vasoconstrictor tone, whereas in the forearm skin this is mediated by vasodilator sympathetic fibres [14]. Previous studies have shown that, when subjects are studied at an ambient temperature of 25°C, vasoconstrictor tone is fully released, as blocking of cutaneous nerves does not result in any further increase in blood flow [15,20].

In conclusion, the present study shows that, during intravital video-microscopy, venous congestion at 60 mmHg for 2 min allows visualization of a maximal skin capillary density on the dorsum of the fingers. Rarefaction of skin capillaries in essential hypertension after maximization with venous congestion strongly suggests that much of the decrease in capillary density in hypertension is due to the structural or anatomical absence of capillaries, rather than to a functional decrease.

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