Impaired retrograde transmission of vasodilatory signals via the endothelium in pre-eclampsia: a cause of reduced tissue blood flow?

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ABSTRACT

There is evidence that tissue blood flow is regulated by retrograde transmission of signals initiated at capillary and post-capillary sites, and transmitted via the endothelium to modulate pre-capillary resistance. We have used pre-eclampsia as a model to test the hypothesis that normal endothelium is required to enable adjustment of blood flow to match tissue requirements. Integrity of the endothelial pathway was assessed by measuring calf blood flow at increasing venous pressures, using an established small cumulative-step venous-congestion plethysmography protocol in ten women with pre-eclampsia, 17 normal pregnant controls and ten non-pregnant women. Endothelial cell activation was assessed by measuring plasma levels of the cell adhesion molecules, intercellular cell-adhesion molecule-1 (ICAM-1), vascular cell-adhesion molecule-1 (VCAM-1) and E-selectin. Baseline calf blood flow was significantly lower in pre-eclampsia than in the other two groups (P < 0.0001; ANOVA). In the pre-eclampsia group, there was a fall in blood flow as venous congestion pressure was raised (P < 0.0001; ANOVA). No such change was observed in the other two groups. A significant inverse correlation was observed between the reduction in blood flow in pre-eclampsia and the levels of E-selectin (r = −0.92, P = 0.0002), VCAM-1 (r = −0.93, P = 0.0008) and ICAM-1 (r = −0.86, P = 0.001). The differences between the pre-eclamptic women and the other two groups support the notion that the failure to sustain blood flow during a cumulative pressure step protocol in the pre-eclamptic group might be influenced by interference with the retrograde transmission of signals via the endothelium in these patients.

INTRODUCTION

Tissue blood flow is influenced by events at the microvascular and postmicrovascular levels of the microcirculation [1]. Pre-capillary resistance, which regulates blood flow through the microvascular bed, is modulated by retrograde transmission of vasodilatory signals via the endothelium from microvascular and postmicrovascular sites [2]. Therefore the vascular endothelium may act as an organ driving vasomotor activity at the pre-capillary...
level, playing an important role in sensing altered local tissue demand and adjusting flow to accommodate these needs [3,4].

It is generally accepted that in the absence of neuronal, hormonal or pharmacological influence, Darcy’s law governs blood flow through parallel circuits of the cardiovascular system. This is described by the Darcy equation: \( Q_a = (P_a - P_V)/R \), where \( Q_a \) is blood flow through a vascular bed, \( R \) is the peripheral vascular resistance (PVR), \( P_a \) is the arteriolar and \( P_V \) is the venous hydrostatic pressure respectively, and \( P_a - P_V \) the arterio-venous pressure difference. Thus, if peripheral resistance (\( R \)) and mean arterial blood pressure (MABP; \( P_a \)) remain constant, blood flow should decrease as venous pressure (\( P_V \)) is increased. Gamble et al. [5] used venous congestion strain gauge plethysmography and Doppler fluximetry to measure lower limb blood flow at increasing venous pressures in non-pregnant healthy controls. They observed that blood flow remained constant even at venous congestion pressures approaching mean arterial pressure. They attributed their results to a progressive reduction of pre-capillary resistance due to retrograde transmission of vasodilatory signals via the endothelium [3,4], and hypothesized that endothelial dysfunction might interfere with this pathway.

Pre-eclampsia, a multisystem disorder of the second half of pregnancy, is characterized by generalized vascular endothelial cell dysfunction [6] and a clinical presentation suggestive of impaired tissue perfusion. Indeed, we have reported [7] that resting calf blood flow is reduced in pregnancies complicated by pre-eclampsia. We have also reported recently [8] that the reduced tissue blood flow might be due to increased post-capillary pressure, resulting from post-capillary margination of leucocytes in pre-eclampsia. However, it is possible that changes in pre-capillary resistance, which is influenced by endothelial-mediated mechanisms, may also play a role in the impaired blood flow [2]. Patients with this condition, therefore, present an ideal human model for testing the hypothesis that an intact endothelial pathway is required for the maintenance of normal tissue blood flow. In the present studies, we have investigated the integrity of the endothelial pathway by measuring blood flow against a background of progressive increases in venous congestion pressure in pre-eclamptics and controls using an established protocol [7]. Previously, we have used plasma levels of the cell adhesion molecules E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell-adhesion molecule-1 (ICAM-1) as markers of endothelial cell activation [8]. We used these markers in the present study in order to test the hypothesis that endothelial cell dysfunction might disrupt retrograde signal transmission and, thereby, contribute to the reduction in blood flow.

METHODS AND MATERIALS

Subjects

We assessed the integrity of the endothelial pathway by measuring resting calf blood flow at increasing venous pressures. We used a small cumulative pressure-step venous-occlusion protocol [5] on ten women with pre-eclampsia, 17 normal pregnant women and ten non-pregnant controls. All the women were matched for age and body mass index (BMI) and the pregnant groups for parity and gestational age. Women with pre-eclampsia were recruited from the antenatal ward, normal pregnant women from the antenatal clinic, and the non-pregnant volunteers were health workers from the Chelsea and Westminster Hospital, London. All the women were non-smokers and were not on any medication. Women with previous or present history of peripheral vascular disease, peripheral neuropathy or any other underlying medical disorders, including oedema, were excluded from the study. Pre-eclampsia was defined according to the criteria of hypertension and proteinuria after 20 weeks of gestation and the reversal of both after the pregnancy. Hypertension was defined as an absolute blood pressure greater than 140 mmHg systolic (SBP) or 90 mmHg diastolic (DBP), taken twice, 6 h apart. Proteinuria was defined as more than 0.5 g/l urinary protein excretion in a 24-h urine sample [9]. The Local Ethics Committee approved the study, and informed consent was obtained from each subject.

Study protocol

Studies were performed in a quiet room at constant temperature (23–24°C). Subjects rested for at least 15 min before the study. Observations were made in the left lateral position, to prevent aorto-caval compression, with the right mid-calf supported at the level of the heart. At each study, arterial blood pressure was measured non-invasively on the ipsi-lateral calf and arm, using a Dinamap Vital Sign Monitor (Type 1800; Critikon, Tampa, FL, U.S.A.). Mean values of SBP and DBP and MABP were calculated from triplicate measurements. Blood flow was estimated using the Filtrass strain-gauge plethysmograph (Filtrass; DOMED, Munich, Germany) [10]. The device, a modification of standard strain-gauge plethysmograph, has been fully described previously [8,10]. Briefly, the congestion pressure cuff, which is attached to a compressor pump built into the apparatus, was placed around the right thigh and enclosed in a rigid corset to reduce filling volume and thus filling time. Changes in calf circumference in response to a rapid increase in cuff pressure were measured using a passive inductive transducer with an accuracy of \( \pm 5 \mu m \). The files were recorded and saved for subsequent ‘off-line’ analysis.
Assessment of blood flow

To measure baseline blood flow, the venous congestion pressure was rapidly raised to 80 mmHg for 10 s and then returned to the original value. We chose 80 mmHg to allow the determination of blood flow at the projected maximum cumulative pressure step of 50 mmHg, without activation of the arterio-venous mechanism described by Henriksen and Sejrsen [5,11]. Blood flow was estimated from the slope of the first 3 s of the volume response to the transient pressure elevation [5]. Blood flow was assessed at each step of a small cumulative-step venous-congestion plethysmography protocol [5,12]. Each small cumulative step of 8 mmHg was maintained for 5 min. It has been shown [5] that the transient elevation of cuff pressure has no longitudinal effect on microvascular parameters such as filtration capacity, which, along with calf venous pressure and other parameters, can be derived from the small cumulative pressure step protocol. The maximum cumulative congestion pressure step did not exceed the DBP. The ratio of calf to brachial SBP was used as an index of normality of the vascular system in both limbs [13]. We used the calf and not the forearm, because the calf is less liable to involuntary movement artefacts.

The values for blood flow at each pressure were normalized by expressing them as a percentage of the control value. We used the Darcy equation, together with the initial values of leg PVR, leg MABP and the values of venous congestion pressure, at each pressure step, to calculate the value of blood flow that would have been expected had the pre-capillary resistance remained constant throughout the procedure. Leg PVR was calculated from blood flow and leg MABP less the venous pressure. These predicted values of blood flow were also expressed as a percentage of the initial values.

Sample collection and analysis

Venous blood samples were obtained from the antecubital vein to assay circulating levels of the cell adhesion molecules E-selectin, VCAM-1 and ICAM-1 (as markers of endothelial cell activation), plasma albumin, total protein, uric acid and creatinine concentrations and also full blood count. Details of the sample collection, storage and assays have been described previously [8].

Statistics

Clinical and blood flow data were normally distributed and are presented as means ± S.D. ANOVA for repeated measures was used to compare the changes in measured and predicted blood flow with venous congestion pressure between the groups of women. Measured and predicted blood flow within each group was also compared using ANOVA. To establish the relationship between the rate of change of blood flow and endothelial function, a linear regression was fitted to the relationship between congestion pressure and blood flow values of all women in each group. The slopes (as summary measures of change of blood flow with congestion pressure) [14] were then calculated and correlated with circulating levels of cell adhesion molecules E-selectin, VCAM-1 and ICAM-1, and clinical parameters. The Statistical Package for Social Sciences (SPSS, version 10) was used for these analyses.

RESULTS

The three groups were similar in age, BMI and, in the pregnant groups, for gestational age (Table 1). Women with pre-eclampsia had significantly higher brachial MABPs (< 0.0001) and mean leg PVRs (< 0.0001), uric acid concentrations (< 0.0001) and haematocrit (< 0.0003), but lower platelet counts (< 0.0001). Babies born to women with pre-eclampsia were significantly smaller compared with those from the normal pregnant controls (P = 0.024), although none of the babies was growth-restricted as assessed by serial ultrasound examination (Table 1).

The calf to brachial arterial SBP ratio was greater than 1.0 in all groups, suggesting the study limbs were all healthy. Plasma albumin and total protein concentrations were significantly lower in the pre-eclampsia group compared with the controls (< 0.0001 for both albumin and total protein; ANOVA). Baseline calf blood flow was significantly reduced in women with pre-eclampsia when compared with normal pregnant and non-pregnant controls (2.47 ± 0.09 compared with 4.29 ± 0.13 and 3.01 ± 0.15 ml · min⁻¹ · 100 ml⁻¹ of tissue respectively; P < 0.0001, ANOVA). However, blood flow was significantly greater in the normal pregnant women (4.29 ± 0.13 ml · min⁻¹ · 100 ml⁻¹) compared with the non-pregnant controls (3.01 ± 0.15 ml · min⁻¹ · 100 ml⁻¹; P < 0.001). Calf blood flow, expressed as a percentage of baseline values in the control groups, did not change significantly as the venous congestion pressure was increased in small cumulative steps to 48 mmHg in either the pregnant or non-pregnant controls. Although the measured blood flows were similar in the normal pregnant and non-pregnant groups (P = 0.65), they were significantly different from the values predicted on the basis of Darcy’s equation (P < 0.0001; Figures 1a and b). In contrast, the measured blood flow in the pre-eclampsia group decreased progressively as the congestion pressure was increased in small cumulative steps to 48 mmHg (Figure 1c). The reduction in blood flow as congestion pressure increased was significantly greater in the pre-eclamptic women than in either of the control groups (P < 0.0001, ANOVA for repeated measures). Moreover, in the pre-eclampsia group, the measured blood flow values were significantly lower than those predicted using Darcy’s equation (P = 0.01, ANOVA for repeated
Clinical and biochemical data for the three groups of women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-pregnant controls</th>
<th>Normal pregnant controls</th>
<th>Pre-eclampsia</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>17</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.0 ± 4.1</td>
<td>31.1 ± 4.0</td>
<td>30.6 ± 7.1</td>
<td>0.62</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.9 ± 0.02</td>
<td>23.1 ± 3.1</td>
<td>25.0 ± 4.4</td>
<td>0.05</td>
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<tr>
<td>Gestational age (weeks)</td>
<td>N/A</td>
<td>37.4 ± 2.0</td>
<td>36.1 ± 3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>N/A</td>
<td>3.42 ± 0.6</td>
<td>2.86 ± 0.4</td>
<td>0.024</td>
</tr>
<tr>
<td>Arm MABP (mmHg)</td>
<td>95.3 ± 5.7</td>
<td>82.6 ± 2.6</td>
<td>105.6 ± 3.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Leg PVR (mmHg·min⁻¹·ml⁻¹·100ml⁻¹)</td>
<td>32.5 ± 5.7</td>
<td>19.6 ± 3.3</td>
<td>44.1 ± 7.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Arm SBP/leg SBP</td>
<td>1.01</td>
<td>1.01</td>
<td>1.02</td>
<td>NS</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.33 ± 0.03</td>
<td>0.31 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>0.0003</td>
</tr>
<tr>
<td>Urates (mmol/l)</td>
<td>0.23 ± 0.04</td>
<td>0.22 ± 0.05</td>
<td>0.38 ± 0.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Platelet count (&lt;10¹⁵)</td>
<td>262.2 ± 46.2</td>
<td>233.5 ± 48.6</td>
<td>139.1 ± 29.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>74.1 ± 3.1</td>
<td>74.3 ± 4.9</td>
<td>62.2 ± 1.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>38.8 ± 4.9</td>
<td>37.8 ± 4.7</td>
<td>23.4 ± 1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E-selectin (pg/ml)</td>
<td>24.3 (19.0–39.6)</td>
<td>26.02 (19.2–40.2)</td>
<td>73.5 (60.3–90.21)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>VCAM-1 (pg/ml)</td>
<td>173.0 (89.0–161.4)</td>
<td>168.3 (101.2–199.6)</td>
<td>355.3 (247.3–423.3)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ICAM-1 (pg/ml)</td>
<td>160.2 (128.3–191.9)</td>
<td>186.3 (149.6–248.1)</td>
<td>209.6 (248.5–363.5)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± S.D., with the exception of E-selectin, ICAM and VCAM, where values are medians (interquartile range). N/A, not applicable; NS, not significant.

We have used the slopes of these relationships as summary measures and the data are summarized in Figure 2 to illustrate the differences between the study groups.

Consistent with our previous observation [8], circulating levels of the cell-adhesion molecules E-selectin, VCAM-1 and ICAM-1, which were used as markers of endothelial cell activation, were significantly increased in pre-eclampsia compared with both normal pregnant and non-pregnant controls. Using the slopes of the flow–pressure relationship as summary measures of peripheral blood flow, we found that they were inversely related to circulating levels of E-selectin ($r = -0.92, P = 0.0002$), VCAM-1 ($r = -0.93, P = 0.0008$) and ICAM-1 ($r = -0.86, P = 0.001$), as well as the plasma uric acid levels ($r = -0.82, P = 0.004$) in the pre-eclampsia group, but not in the controls. No significant correlation was observed between the changes in blood flow and maternal age, BMI, platelet count, MABP, PVR, plasma albumin and total proteins in any of the groups, or with gestational age and birth weight in the pregnant groups.

Within the pre-eclampsia group, circulating levels of cell-adhesion molecules were significantly related to the change in the relationship between congestion pressure and blood flow, i.e. the summary measures ($r = 0.92, P < 0.001$; $r = 0.93, P < 0.001$; and $r = 0.86, P = 0.001$ for E-selectin, VCAM-1 and ICAM-1 respectively; Figure 3). There were no correlations between these parameters within the non-pregnant and normal pregnant groups. Multiple regression analysis of the relationship between blood flow and the circulating levels of cell-adhesion molecules and biochemical markers of pre-eclampsia showed that circulating levels of E-selectin, VCAM-1 and ICAM-1 were independently related to changes in limb blood flow with increasing venous congestion pressure.

### DISCUSSION

The present study has shown that, whereas calf blood flow was maintained as venous congestion pressure increased in normal pregnant and non-pregnant controls, it decreased progressively in women with pre-eclampsia. The studies have also shown that this change in vascular responsiveness is significantly correlated with increases in the plasma levels of cell-adhesion molecules, which are recognized markers of endothelial cell activation [15]. In an earlier study on normal non-pregnant controls [5], the sustained blood flow, as congestion pressure, was raised and was attributed to a progressive reduction in vascular resistance. It was suggested that this was consistent with the hypothesis that upstream arteriolar vasomotor tone is modulated by the retrograde transmission of signals secondary to metabolic activity at the microvascular level [16–21]. The progressive reduction in blood flow in the pre-eclampsia group and its association with markers of endothelial activation is consistent with the notion that activation of endothelial cells might be linked to a failure of this pathway [22]. This is the first evidence that endothelial dysfunction might interfere with the regulating mechanisms for the maintenance of tissue blood flow in man. These findings, together with our previous data [8], suggest that reduced tissue blood flow in pre-eclampsia may be influenced by changes in both post-capillary and pre-capillary resistance.
It is widely recognized that the vascular endothelium plays an important role in vascular changes during pregnancy. The up-regulation of endothelial function during normal pregnancy results in vasodilatation either due to increased vasodilator influences or decreased vasoconstrictor drive [23]. Thus the generalized endothelial dysfunction of pre-eclampsia [24] may play a role in the maternal cardiovascular maladaptation that characterizes the disease. The reduction in baseline calf blood flow in the pre-eclampsia group, compared with controls, supports this hypothesis. The highly significant inverse correlation between the decrease in blood flow with increasing venous congestion pressure (summary measures) [14] and the circulating levels of the cell adhesion molecules E-selectin, VCAM-1 and ICAM-1, as markers of endothelial cell activation in the pre-eclampsia patients, but not the two groups of controls, as illustrated in Figure 3, supports the hypothesis that microvascular retrograde vasodilatory signal conduction may involve endothelial cells [5]. We have observed a significant inverse relationship between serum uric acid levels and changes in blood flow with venous congestion pressure in the pre-eclampsia group, but not the pregnant controls. Although the clinical utility of uric acid as an index of disease severity in pre-eclampsia is not universally accepted [25], we believe that these data strengthen further the case for its acceptance as such. Moreover, we believe these results may indicate that failure of retrograde conduction correlates with both the extent of the endothelial cell activation and the severity of the condition.

This present study addressed neither the transduction mechanism nor the pharmacology of the transmission pathway in these groups of women. However, evidence from studies on animal models, in which the existence of this pathway was established, has suggested several agonists may be involved. These include acetylcholine [26], L-arginine or nitric oxide (NO) [27], integrins [28] and adenosine [29]. The roles played by these agonists in vasodilatory signal conduction in man remain to be established. In a recent study, Anumba et al. [30] suggested that, although the L-arginine/NO pathway may be involved in the vascular changes during normal pregnancy, changes in vascular NO activity are unlikely to be responsible for the impaired vascular reactivity in pregnancies complicated by pre-eclampsia.
but not the pregnant controls, may not reflect a causal relationship. Moreover, it is possible that factors such as vasoconstrictor mediators, levels of which are increased in pre-eclampsia, could have contributed to the impaired vasodilatation observed in the women with pre-eclampsia. Furthermore, it is unlikely that oedema formation, a feature of pre-eclampsia, contributed to the reduced blood flow, since there were no differences in the extent of pitting between the pregnant groups. Since neither plasma albumin nor total protein were significantly reduced in the pre-eclampsia group compared with the pregnant controls, the altered haemorhheology attributable to these changes or the small, but significant, changes in haematocrit, are not likely to have contributed to the observed differences in blood flow in the pregnant groups.

In summary, our present data support the notion that endothelial-mediated retrograde transmission of vasodilatory signals may play an important role in modulating the normal sympathetic pre-capillary vasoconstrictor tone. If this were the case, the generalized endothelial dysfunction of pre-eclampsia could interfere with this conduction pathway, contributing to the observed vasoconstriction. Moreover, this change could exacerbate the haemodynamic consequences of post-capillary cell margination as reported previously [8]. The resulting pre-capillary constriction with reduction in blood flow could lead to the impaired tissue perfusion and the resulting pathologies, which are typical of pre-eclampsia.

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