Relationship between peripheral and coronary function using laser Doppler imaging and transthoracic echocardiography

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ABSTRACT

Vascular dysfunction in the coronary and peripheral circulations is an early prognostic marker of future cardiovascular events. Measurements of coronary and peripheral vascular function in resistance vessels can be made, but rely on invasive procedures, which make them unsuitable for routine application. An assessment of the direct correlation between vascular responses in skin and coronary vessels has not been made previously. In 27 normal healthy subjects (18–55 years of age), we examined the relationship between peripheral and coronary vascular function. Cutaneous perfusion was measured using the non-invasive technique of laser Doppler imaging during iontophoresis of acetylcholine and sodium nitroprusside, and cutaneous vascular conductance was calculated (laser Doppler perfusion/mean arterial pressure). Coronary flow reserve was measured using transthoracic echocardiography during intravenous adenosine infusion. Mean diastolic velocities were measured at baseline and peak hyperaemic conditions from the Doppler signal recordings. CVR (coronary velocity reserve) was defined as the ratio of hyperaemic to basal mean diastolic velocities. There were significant positive correlations between CVR and cutaneous vascular conductance for acetylcholine ($r = 0.399, P = 0.039$) and sodium nitroprusside ($r = 0.446, P = 0.020$). These results support the idea that peripheral measurements of skin blood flow are representative of generalized microvascular function including that of the coronary circulation in normal healthy subjects.

INTRODUCTION

There is compelling evidence for the association between vascular dysfunction in the coronary or peripheral circulation and both atherosclerotic risk and future cardiovascular events [1,2], such as death, myocardial infarction, ischaemic stroke and the need for revascularization procedures [3,4]. The ability to detect changes in areas away from the coronary and cerebral circulation, where the events actually occur, highlights the systemic nature of vascular dysfunction.

Several methods are available for assessing vascular function, such as ultrasound of the brachial artery and pulse wave analysis, but these provide a measure of large vessel function. Assessment of microvascular function is required as it plays an important role in many conditions.
such as end-stage renal disease, diabetes and connective tissue diseases. Laser Doppler studies of the cutaneous circulation have provided valuable information regarding mechanisms of microvascular dysfunction [5,6]. As with any assessment of the peripheral vasculature, the question arises whether such measurements correlate with those in the coronary circulation. Sax et al. [7], using plethysmographic assessments of the forearm, showed that peripheral vascular responses correlated with those in the coronary circulation in patients with microvascular angina. Increased coronary heart disease score is associated with impaired skin microvascular responses, both endothelium-dependent and endothelium-independent [8], and patients with coronary vessel disease have been found to have abnormalities of the cutaneous microcirculation [9,10]. Although associations have been shown between coronary artery disease and skin microvascular function, a direct correlation between vascular responses in the coronary and skin circulation has not been studied in normal healthy subjects.

There has been increasing interest in the non-invasive assessment of coronary flow reserve using TTDE (transthoracic Doppler echocardiography) [11–13]. This technique has been used to measure CVR (coronary velocity reserve) in stenosed and normal epicardial coronary arteries [14–17]. Studies show that the epicardial coronary response to adenosine may be a surrogate marker of coronary resistance vessel dysfunction [18].

The combination of laser Doppler flowmetry and TTDE provides an ideal opportunity to non-invasively examine the direct relationship between peripheral and coronary resistance vessel function in normal healthy subjects. Our hypothesis was that CVR measured using TTDE in subjects with normal epicardial coronary arteries would be related to peripheral microvascular function, measured using laser Doppler imaging.

**MATERIALS AND METHODS**

**Subjects**

A total of 28 healthy non-smoking subjects (18–55 years of age) were recruited from the student and staff population of the hospital. Subjects initially attended for a screening visit to determine their suitability to take part in the study. Written informed consent was obtained at this visit. The study conformed to the standards set by the Declaration of Helsinki, and ethical approval for the study was obtained from the local Ethics Committee. On the day of the study, subjects arrived having refrained from food and drink for at least 3 h and avoided caffeine-containing beverages for at least 12 h.

**Assessment of coronary function**

Measurements of coronary flow velocity were carried out by an experienced echocardiographer, who was blinded to the information on peripheral microvascular function. We have significant experience with this technique [11,14,18]. Imaging of the LAD (left anterior descending) artery and perforating branches and measurement of coronary blood flow was carried out at rest and after intravenous adenosine. A 7.0 MHz transducer (Acuson Sequoia 512; Siemens Medical Solutions) was used. In colour Doppler flow mapping, the velocity range was set in the range of ±12 cm/s. The colour gain was adjusted to provide optimal images. The ultrasound beam was transmitted towards the heart to visualize coronary blood flow in the LAD coronary artery by colour Doppler echocardiography. First, the left ventricle was imaged in the long-axis cross section, and the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal portion of the LAD coronary artery was searched for under the guidance of colour Doppler flow mapping. With a sample volume (2.5 or 3.0 mm wide) positioned on the colour signal in the LAD coronary artery, Doppler spectral tracings of flow velocity in the LAD artery were recorded by fast Fourier transformation analysis. Although we try to align the ultrasound beam direction to distal LAD coronary artery flow as parallel as possible, angle correction is generally needed in each examination because of incident Doppler angle (mean angle, 42°; range, 31–58°). We first recorded baseline spectral Doppler signals in the distal portion of the LAD coronary artery over five cardiac cycles at end-expiration. Intravenous adenosine was then administered (140 μ̇·min⁻¹·kg⁻¹ of body weight) for 2 min to record spectral Doppler signals during hyperaemic conditions. Mean diastolic velocities were measured at baseline and at peak hyperaemic conditions from the Doppler signal recordings. Measurements were then averaged over three cardiac cycles. CVR was defined as the ratio of hyperaemic to basal mean diastolic velocities. The inter- and intra-observer variability for the measurement of coronary Doppler velocity recordings in our laboratory are 4.9 and 4.0% respectively, determined from measurements made in six subjects on two separate occasions.

**Assessment of peripheral vascular function**

Measurements were conducted in a laboratory set at 22 ± 1 °C. Participants were seated comfortably with their arms supported at heart level. Microvascular function was assessed non-invasively in the forearm skin as described by us previously [19]. We measured skin blood flow responses to iontophoresis of ACh (acetylcholine; Sigma) and SNP (sodium nitroprusside; David Bull Laboratories), which are endothelium-dependent and endothelium-independent vasodilators respectively. ACh and SNP were made up as 1% solutions in deionized sterile water and iontophoresed using anodal and cathodal currents respectively. ACh and SNP were delivered using consecutive increases in current: 10, 15, 20, 50 and...
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100 μA, with each current being applied for 4 min. Using large electrodes (internal diameter, 20 mm), this sequence of delivery currents, in our experience, does not cause any non-specific electrical effects. The resulting change in microvascular skin blood flow, termed laser Doppler flux and measured in PU (perfusion units), was assessed using laser Doppler imaging (moorLDI; Moor Instruments). A measure of the overall microvascular response was determined for the total drug delivery period by calculating the AUC (area under the perfusion × time curve) over baseline. Cutaneous vascular conductance was calculated as the AUC divided by mean arterial pressure. The reproducibility of this technique in our hands, determined from repeat measurements on two separate occasions at least 1 day apart in eight subjects, is 11 %.

**Statistical analysis**
Values are expressed as means ± S.D., unless stated otherwise. All data were normally distributed. The univariate association between CVR and peripheral microvascular function was tested using Pearson’s correlations. A P value < 0.05 was considered significant. All analyses were performed using SPSS statistical package (version 13).

**RESULTS**

Satisfactory measurements were obtained in 27 subjects for CVR, and in all 28 subjects for iontophoresis and laser Doppler imaging. Table 1 summarizes the measurements for CVR, laser Doppler imaging and haemodynamic parameters in the 27 subjects that completed both parts of the study. Figure 1 shows a spectral Doppler tracing of the LAD coronary artery flow at baseline and during adenosine-induced hyperaemia. Heart rate increased significantly during adenosine infusion (from 62.7 ± 8.9 to 76.2 ± 14.7 beats/min; P < 0.001), although there was no association between this change and microvascular responses to ACh (P = 0.212) or SNP (P = 0.649). There was no significant change in SBP (systolic blood pressure) and DBP (diastolic blood pressure) during adenosine infusion (Table 1).

There were significant positive correlations between CVR and ACh AUC (r = 0.434, P = 0.024) and SNP AUC (r = 0.362, P = 0.042). Additionally, CVR was positively correlated with both ACh cutaneous vascular conductance (r = 0.399, P = 0.039) (Figure 2) and SNP cutaneous vascular conductance (r = 0.446, P = 0.020) (Figure 3). After adjusting for gender, age and BMI (body mass index), there was a reduced, but still significant, association between CVR and ACh AUC (r = 0.312, P = 0.047) and SNP AUC (r = 0.402, P = 0.042).

**DISCUSSION**

Previous studies have shown an association between the peripheral and coronary circulations, but this correlation has been largely confined to studies that have used invasive procedures [7]. Although laser Doppler imaging can be used non-invasively to assess peripheral microvascular function [19] and provides a good measure of generalized microvascular function [5,6], a direct comparison with coronary resistance vessel function has not been made.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value ± S.D.</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>26.3 ± 10.5</td>
</tr>
<tr>
<td>Gender (n) (male/female)</td>
<td>17/10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.2 ± 7.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.1 ± 12.7</td>
</tr>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>62.7 ± 8.9</td>
</tr>
<tr>
<td>Baseline SBP (mmHg)</td>
<td>124.5 ± 8.8</td>
</tr>
<tr>
<td>Baseline DBP (mmHg)</td>
<td>71.8 ± 8.7</td>
</tr>
<tr>
<td>Baseline mean arterial pressure (mmHg)</td>
<td>85.1 ± 14.7</td>
</tr>
<tr>
<td>Peak heart rate during adenosine (beats/min)</td>
<td>76.2 ± 14.7</td>
</tr>
<tr>
<td>SBP after adenosine (mmHg)</td>
<td>122.7 ± 11.1</td>
</tr>
<tr>
<td>DBP after adenosine (mmHg)</td>
<td>72.2 ± 8.1</td>
</tr>
<tr>
<td>Baseline diastolic velocity (m/s)</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>Peak diastolic velocity during adenosine (m/s)</td>
<td>0.85 ± 0.27</td>
</tr>
<tr>
<td>Coronary flow velocity reserve</td>
<td>3.73 ± 0.72</td>
</tr>
<tr>
<td>ACh AUC</td>
<td>1796 ± 169</td>
</tr>
<tr>
<td>SNP AUC</td>
<td>1190 ± 121</td>
</tr>
<tr>
<td>ACh cutaneous vascular conductance (mmHg·min⁻¹)</td>
<td>21.4 ± 2.1</td>
</tr>
<tr>
<td>SNP cutaneous vascular conductance (mmHg·min⁻¹)</td>
<td>13.8 ± 1.3</td>
</tr>
</tbody>
</table>

Figure 1 Spectral Doppler tracing of the LAD coronary artery flow at baseline (left-hand side) and during adenosine-induced hyperaemia (right-hand side)
Figure 2 Correlation between coronary flow velocity reserve during adenosine infusion and the cutaneous vascular response to ACh in 27 subjects

\[ r = 0.399, P = 0.039. \]

Figure 3 Correlation between coronary flow velocity reserve and the cutaneous vascular response to SNP in 27 subjects

\[ r = 0.446, P = 0.020. \]

Very few studies have tested the direct association between resistance vessels in the peripheral and coronary circulations of normal healthy subjects, mainly because it has not been possible to measure coronary function in subjects in whom coronary angiography is not indicated.

Our present findings that vascular responses in the forearm skin microvessels correlate with CVR support the idea that the function of peripheral and coronary resistance vessels is indeed related, and that the assessment of peripheral vascular function using laser Doppler imaging can provide an indication of coronary vascular function, at least in healthy subjects. Further studies are needed to determine whether this relationship holds for subjects with established microvascular disease. Our present findings in normal subjects contrast with those of Böttcher and co-workers [20], who found no association, perhaps because the techniques for assessing the peripheral resistance vessels were different in the two studies. Additionally, differences in the size of vessels studied by Böttcher et al. [20] (coronary resistance vessels compared with brachial artery function) may have accounted for this discrepancy.

In the present study, we used TTDE to measure coronary flow reserve, which has been shown to accurately reflect similar measurements obtained using an invasive Doppler guide wire [12]. Without estimation of the coronary artery diameter, the technique only allows measurement of coronary flow velocity, but not changes in coronary flow. However, it has been shown that coronary flow reserve measured using both parameters is closely related [11]. In the absence of obstructive coronary artery disease, CVR in response to adenosine infusion provides a measure of the function of coronary resistance vessels. A reduced coronary flow reserve has been reported in women with chest pain [21], in young female patients with systemic lupus erythematosus [18] and in patients with systemic sclerosis [22], all in the absence of obstructive coronary artery disease. Coronary flow reserve may be abnormal when the resistance vessels are compromised by left ventricular hypertrophy, coronary endothelial dysfunction or other diseased rheological conditions.

Although studies using laser Doppler flowmetry do not conform to a single standardized protocol, making between-study comparisons complex, it is nevertheless a relatively simple technique to use, which suffers less from user dependency and is technically less challenging than other methods, such as assessment of brachial artery reactivity [23]. The combination of laser Doppler flowmetry and iontophoresis has been used successfully by us and other researchers to demonstrate microvascular dysfunction in several conditions [19,24–26], and the good reproducibility of the technique has been confirmed [24,27]. Importantly, changes in skin microvascular function are detectable in children with risk factors for cardiovascular disease well before clinical presentation of symptoms [19,28], thus making laser Doppler flowmetry a useful potential tool for the early detection of cardiovascular risk. Additionally, subtle changes in skin microcirculation can be detected following therapeutic intervention [29].

Results from other studies also demonstrate that peripheral assessments of skin microvascular function do relate to coronary vascular function and coronary heart disease risk. Jadhav et al. [25] found that skin microvascular responses were markedly impaired in patients with cardiac syndrome X, who have angiographically normal coronary arteries, and also demonstrated the predictive value of the technique by showing an odds ratio for cardiac syndrome X of 7.38. Skin microvascular responses are decreased in patients with angiographically demonstrated coronary artery disease [10] and with higher cardiovascular risk scores in healthy individuals [30].

A limitation of the present study is the relatively small sample size and a stronger association might have been achieved with a larger sample size. On the basis of
our correlation, approx. 16–20% of the change in one variable could be explained or accounted for by a change in the other variable. Although we accounted for confounders, such as age, gender and BMI, other confounding cardiovascular risk factors were not accounted for, and the lack of these might explain the relative weakness of the association.

We also did not obtain a measure of resistance vessel structure such as minimal vascular resistance, which can be determined by measuring the blood flow response to maximal reactive hyperaemia. We did not measure this in the present study because the laser Doppler imager does not have the required speed to accurately measure this response. Furthermore, the main purpose of the present study was to assess the association of coronary function with that of a commonly used method for assessing the skin microvascular response, and one that shows good reproducibility using the imager system. Using a single-point laser Doppler flowmeter which continuously measures skin blood flow, but from a relatively very small sample area compared with the imager, we have shown in our pilot studies in 20 normal subjects (E. Khan and J. J. F. Belch, unpublished work) that the skin maximal hyperaemic response is highly correlated with laser Doppler imager measurements of skin vascular responses to ACh ($r = 0.84, P < 0.0001$) and SNP ($r = 0.77, P < 0.0001$). Thus we believe it is likely that the maximal skin hyperaemic response would also correlate with CVR.

Our association between peripheral skin and coronary vascular function only applies to healthy subjects and we cannot extrapolate these findings to patient populations. In normal healthy subjects it is assumed that changes in flow velocity reflect coronary microvascular function and are not related to epicardial disease, which of course might not be the case in an older population with risk factors for coronary artery disease. Nevertheless, establishing this direct association between skin and coronary vasomotor function in normal subjects is important because it lends support to the use of the skin as a useful vascular bed to study normal physiological control mechanisms, which are required in order to understand how the microcirculation becomes altered in disease states.

Invasive techniques have been proven to be sensitive and specific for the assessment of cardiovascular risk, but have limited practical value for routine use. Laser Doppler imaging has the advantage of being relatively easy to use with less operator dependency than other techniques and is less expensive. Additionally, its completely non-invasive nature makes it well suited for studies in all age groups, including young children and infants.

ACKNOWLEDGMENTS

This study was supported by the Medical Research Council, UK and TENOVUS Scotland. We thank Madeleine Swann and Dr Yvonne Fogarty for technical assistance, and Elaine Carr for subject recruitment.

REFERENCES


Received 5 December 2007/27 February 2008; accepted 13 March 2008
Published as Immediate Publication 13 March 2008, doi:10.10142/CS20070431