Aims To validate a novel method for assessment of coronary endothelium-dependent microvascular function and compare this index with the adenosine-derived coronary flow reserve (CFR).

Methods and results We validated use of intra-coronary pressure wire-derived thermodilution to assess changes in coronary flow compared to Doppler flow-wire/quantitative coronary angiography (QCA) derived data in response to the endothelial agonist substance-P (endothelium-dependent response). There was a close correlation between Doppler/QCA- and thermodilution-derived assessment of endothelium-dependent microvascular function ($r = 0.76; P < 0.001$). Next, pressure wire-based thermodilution was employed to sequentially compare CFR (hyperaemia achieved with adenosine-140 μg/kg/mL) with changes in coronary flow in response to substance-P (20 pmol/min intra-coronary infusion; 2 min) in 65 unobstructed coronary arteries. There was no correlation between CFR and coronary endothelium-dependent microvascular response ($r = 0.08; P = 0.50$). Both indices were in turn compared with clinical markers of endothelial dysfunction, namely Framingham risk score (FRS—a marker for cardiovascular risk factor clustering, hence an indirect clinical measure of endothelial dysfunction) and presence/absence of diabetes. Patient’s FRS correlated with coronary endothelium-dependent microvascular response ($r = 0.48; P < 0.001$), but not with CFR ($r = 0.14; P = 0.25$). Diabetic patients had greater endothelial dysfunction than non-diabetics ($P < 0.001$) whereas CFR was not influenced by diabetes ($P = 0.10$).

Conclusion A simple pressure wire-based thermodilution technique can be used to assess coronary endothelium-dependent microvascular function. Adenosine-derived CFR does not adequately interrogate the endothelium-dependent component of coronary microvascular function.
A comprehensive assessment of the coronary microcirculation requires information on both the endothelium-dependent and -independent coronary microcirculatory responses. Adenosine-derived CFR, which achieves maximal hyperaemia primarily through its vasodilator action on vascular smooth muscle cells, is potentially an incomplete measurement of coronary microcirculatory function, due to its inability to distinguish between endothelium-dependent and -independent microcirculatory responses. Furthermore, invasive assessment of human coronary endothelium-dependent microvascular function is rather cumbersome and technically challenging and therefore rarely used outside a research setting. Such an assessment requires calculation of changes in total coronary flow after local infusion of endothelial agonists, using an intra-coronary Doppler wire and quantitative coronary angiography (QCA).

The purpose of this study was to validate a novel, simple technique for rapid assessment of human endothelium-dependent coronary microvascular function using the intra-coronary pressure wire, and thermodilution. We also sought to compare adenosine-derived CFR with changes in blood flow in response to the specific endothelial agonist, substance-P, in unobstructed coronary arteries of patients with varying degrees of endothelium-dependent and -independent microvascular dysfunction.

Methods

Between July 2004 and February 2006, 222 consecutive patients with stable, single- or two-vessel coronary artery disease undergoing elective percutaneous coronary intervention (PCI) were screened for inclusion in the study. Seventy-seven patients fulfilled the inclusion/exclusion criteria. Two patients refused consent for inclusion in the study and 10 patients who gave informed consent did not undergo coronary measurements secondary to technical problems and time limitations in the catheterization laboratory. The remaining 65 patients were recruited to the study. Results form all patients who underwent coronary measurements were included in the final analysis.

Each patient underwent detailed characterization on the basis of anthropomorphic data, Framingham Risk Score (FRS), fasting lipid and glucose levels, and assessment of coronary endothelium-dependent microvascular function and CFR. Coronary studies were performed in an angiographically unobstructed artery prior to PCI to an adjacent artery [23 in the left anterior descending (LAD), 30 in the left circumflex (LCx), and 12 in the right coronary artery (RCA)]. All vasoactive drugs were discontinued >24 h prior to the study. Patients with >50% left main stem coronary disease, previous bypass surgery, left ventricular impairment, valvular heart disease, poorly controlled airways disease (a contraindication to administration of adenosine), and significant renal, hepatic, or inflammatory disease were excluded. The study had local ethics committee approval and all patients gave written informed consent prior to recruitment to the study.

Anthropomorphic and biochemical measurements

Anthropomorphic measurements [weight (kg), height (m), body mass index (BMI kg/m²)] were made according to published guidelines. All subjects had blood taken after an overnight fast for full lipid profile [total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, and high density lipoprotein (HDL) cholesterol] and glucose levels immediately before cardiac catheterization. Blood samples were analysed in the hospital biochemistry laboratory.

Framingham risk score

FRS was calculated on the basis of a number of categorical variables (age, total cholesterol, HDL cholesterol, systolic/diastolic blood pressure, presence/absence of diabetes, and smoking history) using previously published algorithms.

Cardiac catheterization protocol

Coronary endothelium-dependent microvascular function and then CFR were measured (always in that order) prior to PCI in each study artery. Measurements taken in this order ensured that administration of intra-coronary nitrates for assessment of CFR did not interfere with coronary endothelial responses. At least 5 min were allowed to elapse between each measurement to ensure return of coronary blood flow to baseline. Procedures were covered with 5000 units of un-fractionated heparin. 6F-guiding catheters were used to intubate coronary ostia.

Assessment of coronary endothelium-dependent microvascular function

Accurate invasive assessment of coronary microvascular endothelial function is dependent on calculating the maximal percentage change in coronary blood flow from baseline in response to an endothelial agonist. Intra-coronary Doppler and QCA are currently used to accurately assess changes in coronary blood flow. We developed a method for assessment of coronary microvascular endothelial function using a thermodilution technique. Previous experimental and human studies have demonstrated that the transit time (Tmn) of an intra-coronary injectate of room-temperature saline derived from thermodilution curves is inversely proportional to coronary flow. Therefore, it can be assumed that a percentage decrease in Tmn represents a percentage increase in coronary flow. We used an intra-coronary pressure/temperature sensor-tipped guide wire (Radi pressure wire 4–Radi Medical Systems, Uppsala, Sweden), which has an accuracy of 0.05 C within a temperature range of 15–42 C, to derive thermodilution curves.

In the first 20 patients recruited to the study, we sought to validate the use of thermodilution to assess coronary endothelium-dependent microvascular function against the existing ‘gold standard’ of Doppler flow-wire and QCA by dually assessing changes in coronary flow using both Doppler/QCA (as described previously), as well as thermodilution (using the Radi pressure wire 4) in each study artery. Having established a close correlation between the two techniques, the thermodilution technique was then adopted as the sole means of assessment for microvascular endothelial function in the subsequent 45 subjects as described below.

Validation of the thermodilution technique for assessment of endothelium-dependent microvascular function

A Radi pressure wire was calibrated and advanced to the tip of the guiding catheter for equalization of pressure/temperature signals. The wire was then introduced into the distal third of the study coronary artery. All lines were flushed with normal saline to avoid injection of contrast medium. Thermodilution curves were obtained (in triplicate) from a hand-held, 3 mL, brisk injection of room temperature normal saline at baseline and at maximal flow after a 2 min intra-coronary infusion of substance-P (Merck Biosciences Ltd, UK–20 pmol/min, equating to an infusion rate of 1 mL/min). Substance-P was infused via the guide catheter using a high-speed infusion pump to ensure an accurate rate of infusion. The same dose of substance-P was used in all three coronary arteries. Mean Tmn (at baseline and maximal flow) was derived online using dedicated software within the Radi-Analyzer unit. The percentage change in coronary blood flow was calculated offline from the ratio of mean baseline and maximal Tmn values. The dose response relationship for substance-P in human coronary arteries has been well characterized and our choice of dose was...
based on this literature as well as our previous experience with this agent.16,17

At least 5 min were allowed to elapse for coronary blood flow to return to baseline before introduction of a Doppler guide-wire (Flowire, Cardiometrics Inc., Mountain View, CA, USA) into the study artery. The Doppler crystal was placed at the level of the distal sensor on the Radi wire. The Doppler wire was manipulated to obtain optimal traces. Continuous average peak velocity (APV) Doppler traces were recorded at baseline followed by angiographic images of the study artery in two orthogonal views for measurement of coronary arterial diameter. After a 2 min intra-coronary infusion of substance-P (20 pmol/min), continuous APV Doppler traces were recorded at maximal coronary blood flow. The two orthogonal angiographic views were repeated immediately after recording maximal APV traces to obtain maximal coronary artery diameter. Changes in the diameter of the study artery were calculated using automated Philips QCA edge detection system in a 2.5-5 mm length segment approximately 2.5 mm distal to the tip of the Doppler wire. Absolute coronary blood flow was calculated as previously described from the product of corresponding peak APV values and QCA-derived coronary artery diameter (1/2 × APV × coronary cross-sectional area) at baseline and maximal flow.13 The percentage change in coronary blood flow was obtained from the ratio of baseline and maximal blood flow values. To minimize bias from any potential tachyphylaxis to substance-P, the thermodilution and Doppler techniques were alternately used as the first protocol in the initial 20 patients.

Assessment of coronary flow reserve

CFR was assessed using a thermodilution technique as previously described.12,13,15 As already outlined, this technique is based on the principle that the thermodilution transit time (Tmn) of an intra-coronary injectate of room temperature saline is inversely proportional to coronary blood flow.12,13

A Radi wire pressure was prepared and advanced to the distal third of the study artery to obtain thermodilution curves (as described above). All lines were again flushed with normal saline to avoid injection of contrast medium. Two hundred microgram of intra-coronary glyceryl trinitrate (GTN) was then administered prior to any measurement.13,15 Triplicate thermodilution curves were obtained at baseline and at maximal steady-state hyperaemia [achieved by infusion of 140 μg/kg/min of adenosine (Adenoscan, Sanofi-Synthelabo, Surrey, UK) via the femoral vein].13 Mean Tmn (at baseline and maximal hyperaemia) and CFR were derived online using dedicated software within the Radi-Analyzer unit. It has been demonstrated that this method for the assessment of CFR may be technically less challenging with greater reproducibility than the traditional invasive method for assessment of CFR using the intra-coronary Doppler flow-wire.11,13,15

Statistical analysis

Continuous variables were presented as mean ± SEM. Unpaired t-test was used to analyse differences in continuous variables. ANOVA was used to assess the relation between the mean percentage change in blood flow in different coronary arteries. The variability of Tmn values between a set of three measurements was investigated to give a power of 90% (alpha 0.05) to detect a significant difference between the proposed Doppler and thermodilution techniques for the assessment of endothelium-dependent microvascular function. Linear regression analysis was used to compare thermodilution- and Doppler-derived endothelium-dependent microvascular function, CFR and endothelium-dependent microvascular function and CFR/endothelium-dependent microvascular function and FRS. A Bland-Altman plot was constructed to illustrate the level of agreement between the established Doppler/QCA method and our proposed thermodilution-derived assessment of endothelium-dependent microvascular function.19 The plot demonstrates the relationship between the average value between Doppler/QCA and thermodilution-derived percentage change in blood flow and the absolute difference between Doppler/QCA and thermodilution-derived percentage change in blood flow.19 Statistical significance was accepted at P < 0.05.

Results

Sixty-five patients undergoing single/two vessel PCI were recruited and a single unobstructed coronary artery studied in each patient. Baseline characteristics of patients are summarized in Table 1. The mean age of the patients was 64 ± 1 years (31 (51%) male). Ten (15%) patients were diabetic.

Thermodilution curves were obtained in triplicate for all measurements. The variability of Tmn values (derived from thermodilution curves) within a set of three measurements at baseline and maximal flow/hyperaemia for assessment of both endothelium-dependent microvascular function (P = 0.11) and CFR (P = 0.87) were similar.

Substance-P exerted no systemic haemodynamic effects (mean systemic blood pressure—baseline: 93 ± 2 mmHg, post substance-P 88 ± 2 mmHg; P = 0.10). Although, the same dose of substance-P was used in all three coronary arteries, the mean percentage change in flow was similar in all three coronary arteries (F = 1.88, P = 0.16, data not shown).

Comparison of thermodilution- and Doppler flow-wire/quantitative coronary angiography techniques for assessment of coronary endothelium-dependent microvascular function

Coronary endothelium-dependent microvascular function was assessed simultaneously using thermodilution and

| Table 1 Baseline characteristics of patients (mean ± SEM) |
|------------------|------------------|------------------|
| Age (years)      | 64 ± 1           | Male (%)         | 31 (51%) |
| Cardiovascular risk factors | Current smoker (%) | 24 (37%) |
| Hypertension (%) | 44 (68%)         | Diabetes (%) | 10 (16%) |
| Abnormal lipid profile (%) | 55 (85%) |
| Family history (%) | 25 (38%) |
| Systolic blood pressure (mmHg) | 136 ± 3 |
| Diastolic blood pressure (mmHg) | 73 ± 2 |
| Height (m)       | 1.7 ± 0.01       | Weight (kg)     | 79.1 ± 2.4 |
| Body mass index (kg/m²) | 27.0 |
| Lipid profile    |                  | Total cholesterol (mmol/L) | 3.5 ± 0.1 |
| LDL cholesterol (mmol/L) | 2.0 ± 0.1 |
| HDL cholesterol (mmol/L) | 1.0 ± 0.04 |
| Triglycerides (mmol/L) | 0.8 ± 0.1 |
| Glucose (mmol/L)  | 4.9 ± 0.2        |
| Study artery     |                  | RCA (18%)        |
| LAD (36%)        |
| LCx (46%)        |

LDL, low density lipoprotein; HDL, high density lipoprotein; RCA, right coronary artery; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery.
Doppler/QCA in the first 20 study vessels. The mean percentage change in absolute coronary blood flow measured by Doppler/QCA \[31.9 \pm 3.6 \text{ (range: 5.8–70.0)}\] and the percentage reduction in Tmn \[33.3 \pm 3.8 \text{ (range: 5.5–66.0)}\], which is inversely proportional to coronary flow, were similar \(P = 0.79\). As demonstrated in Figure 1A, there was a close correlation between percentage changes in coronary blood flow as measured by the two techniques \((r = 0.76, P < 0.001)\). The corresponding Bland-Altman plot is shown in Figure 1B. The average absolute difference between Doppler/QCA and thermodilution-derived percentage change in blood flow was 8.9 \(\pm\) 1.6% (range: 1.5–23.5%).

Association between coronary flow reserve and coronary endothelium-dependent microvascular function

Thermodilution-derived CFR and coronary endothelium-dependent microvascular function (both expressed as ratio of Tmn derived from maximal to baseline coronary blood flow) were compared in 65 coronary arteries. Mean adenosine-derived CFR was 3.15 \(\pm\) 0.21 and substance-P induced change in blood flow was 1.44 \(\pm\) 0.07. As outlined in Figure 2, there was no correlation between CFR and coronary endothelium-dependent microvascular response \((r = 0.08, P = 0.50)\).

Association between coronary flow reserve, coronary endothelium-dependent microvascular function, and clinical markers of endothelial function

Traditional cardiovascular risk factors are known to be associated with peripheral and coronary endothelial dysfunction.\(^1\) Using each patient’s FRS as a marker for global cardiovascular risk factor clustering\(^8,10\) and hence an indirect indicator of endothelial dysfunction, we demonstrated that there was a close correlation between coronary endothelium-dependent microvascular function and FRS \((r = -0.48, P < 0.001)\), but no association between CFR and FRS \((r = 0.14, P = 0.25)\) (Figure 3).

Diabetes is a further clinical indicator of endothelial dysfunction.\(^1,2\) As expected, in our group of patients diabetics had significantly greater coronary endothelium-dependent microvascular dysfunction \(\%\) change in coronary blood flow–diabetics: 7.7 \(\pm\) 2.2, non-diabetics: 26.5 \(\pm\) 2.6, \(P < 0.001)\). Although CFR values were lower in diabetics \(2.4 \pm 0.4\) in comparison to non-diabetics \(3.3 \pm 0.2\), the differences did not reach statistical significance \((P = 0.10)\) (Figure 4).

Discussion

Coronary blood flow is regulated by a complex interaction between endothelium-dependent and -independent...
vascular function at the level of resistance vessels (<400 μm in diameter) in the heart. Responsiveness of these vessels to specific agonists, assessed by changes in coronary blood flow, forms the basis for investigating coronary microcirculatory function.

In a clinical setting agonists such as adenosine (as in measurement of CFR) are often used to assess overall microcirculatory function on the basis of their ability to induce maximal vasodilatation of coronary resistance vessels. Vasodilatation is primarily achieved through direct effects of adenosine on vascular smooth muscle cells.1,7 There is also evidence that a small proportion of the maximal vasodilatory response may involve the release of nitric oxide (NO) from endothelial cells (through direct action of adenosine on endothelial cells and/or indirectly via flow-mediated dilatation).1,7 Other agonists such as dipyridamole and papaverine, which can also be used for the assessment of coronary microcirculatory function, induce maximal hyperaemia in a similar fashion to adenosine largely through direct smooth muscle vasodilatation. In view of the potential influence of adenosine on both the endothelium-dependent and -independent components of the microcirculation, well-recognized methods for specific assessment of the endothelium-dependent microvascular function using endothelial agonists such as substance-P or acetylcholine (Ach)20 are rarely undertaken outside the context of research protocols.

The results of the present study show that measurement of adenosine-derived CFR alone does not provide satisfactory or sensitive information about overall human coronary microvascular function. Our results demonstrated no correlation between CFR and more specific (substance-P-induced) measures of endothelium-dependent microvascular function in a cohort of 65 patients with coronary artery disease. Previous studies have demonstrated endothelial dysfunction in the presence of cardiovascular risk factors (such as clinical diabetes)1,2 and in association with increasing score on algorithms calculating global cardiovascular risk (such as the FRS).21–23 Our study demonstrated a similar association between specific assessment of coronary endothelium-dependent microvascular function using substance-P and FRS/clinical diabetes. In contrast, we found no significant correlation between adenosine-derived CFR and FRS. Furthermore, unlike previous studies demonstrating a reduced

Figure 3  Correlation between coronary endothelium-dependent microvascular function [(A)–(endothelial function)–expressed as percentage change in blood flow] and coronary flow reserve [(B)–expressed as ratio of hyperaemic to baseline transit time] and Framingham risk score.

Figure 4  Differences in endothelium-dependent microvascular function [(A)–(endothelial function)–expressed as percentage change in blood flow] and coronary flow reserve [(B)–expressed as ratio of hyperaemic to baseline transit time] in patients with and without diabetes.
Thermodilution technique to assess coronary endothelium-dependent microvascular function

CFR in subjects with cardiovascular risk factors, the differences in CFR between diabetics and non-diabetics in our study did not reach statistical significance. While it is possible that this finding may be secondary to a type B error, this finding may also be an indicator that endothelial-specific agonists such as substance-P (in preference to adenosine, which has a less predictable influence on the endothelium) yield more refined data when assessing the microcirculatory influence of factors that primarily influence vascular function (such as diabetes) through their actions on the endothelium. We propose that a more comprehensive assessment of the coronary microcirculation requires separate interrogation of both the endothelium-dependent and-independent vasomotor responses. Although, it is known that the response of a coronary artery to an endothelial agonist can be heterogeneous, the pharmacokinetics of substance-P has been well characterized in the human coronary circulation. Other agonists including adenosine will also have similar limitations. Moreover, use of the pressure wire as in our technique allows for correction of flow values by giving simultaneous fractional flow reserve (FFR) for the study vessel.

In the healthy endothelium, agonists such as substance-P and Ach vasodilate coronary resistance vessels by stimulating release of NO from the endothelium. However, in the context of a dysfunctional endothelium (as in the presence of atherosclerosis or cardiovascular risk factors) the vascular response to substance-P and Ach can vary. Unlike substance-P, Ach has been shown to have a paradoxical vasoconstrictory action on coronary vessels in a proportion of patients with a dysfunctional endothelium. This action is induced by direct (and unopposed) stimulation of the vascular smooth muscle muscarinic receptors by Ach. Considering all patients recruited to our study had atherosclerotic changes, we felt the paradoxical vasoconstrictive influences of Ach would be inappropriate in our patient population. Hence substance-P was used as the endothelial agonist of choice in our study.

The thermodilution technique described in our study is quick, reproducible, and can easily be applied by interventional cardiologists. While both the traditional technique of Doppler flow-wire/QCA and the current method involve instrumentation of the artery with a single guide wire and the use of an intra-coronary agent, we believe the technique described in this paper offers several advantages. The pressure wire is widely used by interventional cardiologists around the world and the majority of catheter laboratories are familiar with both its use and the analyser with which it interfaces. Pressure and thermodilution data are highly reproducible and in sharp distinction with the Doppler flow-wire, do not depend on the precise positioning of the wire tip within the vessel. Flow velocity data acquired from a Doppler flow-wire are notoriously sensitive to wire position and respiratory artefact. Moreover, QCA is a crude way of calculating vessel cross-sectional area in order to establish true flow measurements and depends on the analysis package, site of the vessel at which measurements are taken and magnification artefact from the imaging equipment. The intra-coronary pressure wire is also significantly cheaper (at around 500 Euro) in comparison to the Doppler flow-wire (at around 800 Euro) and its use does not incur the indirect costs involved in the purchase of specific QCA soft/hardware. Furthermore, the use of an intra-coronary pressure wire has the added advantage of allowing assessment of several potentially clinically useful indices including FFR, CFR, and endothelium-dependent microvascular response without the need for repeated coronary instrumentation. The intra-coronary pressure wire can also be used as a guide wire for possible PCI in the same patient. The multiple applicability of the intra-coronary pressure wire in assessing a number of physiological indices and as a PCI guide wire are both additional major indirect cost saving advantages of our proposed technique.

Study limitations

The original mathematical validation of thermodilution-derived CFR is based on the fundamental assumption that the volume of the epicardial study vessel remains constant at baseline and maximal hyperaemia. Clinically, this is achieved by administration of intra-coronary GTN as outlined in our study protocol. This assumption cannot be made when assessing coronary endothelial function using a thermodilution technique because administration of nitrates prior to infusion of substance-P would interfere with the endothelial response. Despite this potential limitation, the results from the validation section of our study with 20 patients demonstrated a close correlation between thermodilution-derived changes in coronary blood flow and the current gold standard using intra-coronary Doppler and QCA. Two of our thermodilution CFR values were very high (CFR values of 8.7 and 9.6). As indicated these findings were not explained by differences in percentage variability between thermodilution-derived Tmm values at baseline and maximal hyperaemia post adenosine infusion. Furthermore, the use of a weight-adjusted systemic infusion of adenosine ensured that a maximal steady-state hyperaemia was reached all coronary territories. In a small proportion of cases, thermodilution CFR has been shown to overestimate the true value of CFR. This may be one explanation for our observations. Although, we do not have a satisfactory explanation for this, such artefacts have been previously described with intra-coronary thermodilution techniques.

Conclusions

We have validated a quick and reliable method for assessing human coronary endothelium-dependent microvascular function using a thermodilution technique with the endothelial agonist, substance-P. This measure of endothelial microvascular function correlates well not only with the current ‘gold standard’ technique of Doppler wire/QCA, but also with clinical markers known to be associated with endothelial dysfunction. Furthermore, the present study indicates that adenosine-derived CFR does not reliably interrogate the endothelium-dependent component of coronary microcirculatory vasodilator function. The utility of applying this pressure wire-based method for measuring coronary microvascular endothelial function to larger groups of patients undergoing treatment in the cardiac catheterization laboratory, for example with respect to clinical outcomes, merits investigation in further studies.
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