Aspects of endothelial function testing in coronary vascular disease

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Exercise-induced ischemia after successful PCI is related to distal coronary endothelial dysfunction

submitted for publication

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ABSTRACT

BACKGROUND As endothelial dysfunction can be responsible for myocardial ischemia even in the absence of significant coronary lesions, we aimed to assess the correlation between endothelium-dependent vasomotor function and inducible ischemia late after successful coronary angioplasty.

METHODS In 30 patients without angiographic restenosis or coronary disease progression, coronary endothelial function was determined by acetylcholine infusion, 6 months after elective single-vessel stenting of the left coronary artery. Acetylcholine-induced diameter changes were assessed in the proximal and distal segments, both of the stented and the contra-lateral vessel, by means of quantitative coronary angiography. A maximal workload ergometric test was also performed prior to endothelial function testing.

RESULTS Acetylcholine induced significant vasoconstrictive responses in the distal, but not in the proximal segments, both of the stented (-11±7% vs. baseline; p<0.01) and the contra-lateral vessel (-11±6%; p<0.01), which were significantly correlated (R=0.48; p<0.05) and were completely reverted by nitro-glycerine. Inducible ischemia was the only predictive factor for distal vasoconstriction in the stented (p<0.01) but not in the contra-lateral vessel (p=0.06). Patients with minor signs of ischemia at the ergometric test showed a greater vasoconstriction than those with a completely normal tests (-16±7% vs. -7±6%; p<0.01).

CONCLUSIONS Exercise-induced ischemia late after successful PCI is related to distal coronary endothelial dysfunction.
EXERCISE-INDUCED ISCHEMIA RELATED TO CORONARY ENDOTHELIAL FUNCTION

INTRODUCTION

Inducible myocardial ischemia can be frequently detected also in the absence of significant coronary stenosis (1) and after successful revascularisation procedures (2). Endothelial dysfunction has been held responsible for impaired coronary vasomotor function, cardiac adverse events and myocardial ischemia, also in the presence of mild coronary artery disease (3-6). Percutaneous coronary interventions (PCI) have proven to be an effective treatment for occlusive coronary disease, and, among all the available techniques, coronary stenting is by far the best in obtaining long-term clinical and angiographic results (7;8). However, evidences of persistent endothelial dysfunction in reperfused coronary vessels have been provided both after successful balloon angioplasty (9) and stenting (10). Aim of this experimental study was to assess the impact of endothelial dysfunction on inducible ischemia in a stented and contralateral coronary vessel in the absence of angiographically detectable coronary artery disease late after successful stenting.

METHODS

Patients’ selection. Consecutive patients submitted to elective percutaneous coronary intervention with stent placement for the presence of a flow-limiting stenosis (lumen reduction in percentage >80%) in a single vessel disease involving a major branch of the left coronary artery were asked permission for re-catheterisation and a provocative test with acetylcholine for endothelial function assessment at 6 months follow-up. Exclusion criteria were severe systemic hypertension, previous myocardial infarction, primary stenting or small vessels (≤ 2.5 mm), or left bundle block at basal ECG. Stenting procedures had been performed accordingly to conventional techniques and under intravascular ultrasound guidance in order to achieve an optimal post-deployment result. Inclusion criteria for the follow-up investigation were: low (0-1) Canadian Cardiovascular Society (CCS) class angina at follow-up and suitability for acetylcholine provocation testing. Three days before the endothelial function measurement, all patients were asked to discontinue any vasoactive medication, and the day before re-catheterisation they underwent a maximal workload test at bicycle ergometry. The study protocol was approved by our Institutional Review Board and a written informed consent was obtained from all patients.
Signs of ischemia during bicycle ergometry. Standard exercise stress testing was performed by upright bicycle ergometry, beginning at a workload of 50W and increasing in 10 W increments every 30 seconds. Exercise was terminated if the patients was unable to maintain a cycling frequency > 50 rpm due to physical exhaustion, angina or dyspnoea, or evidence of myocardial ischemia with documented planar or down sloping ST-segment depression, with both major and minor signs of inducible ischemia (11).

Coronary angiography. The angiographic study was performed by femoral approach with 6F common diagnostic catheters (Cordis BV, Roden, The Netherlands). Selective coronary angiograms were acquired in at least two orthogonal projections, both for the left and the right coronary artery. On-line quantitative coronary analysis was then performed, using a validated computerized system (CMS, Medis Co., Nuenen, the Netherlands), to assess: reference lumen diameter (rld), minimal lumen diameter (mld) and lumen reduction percent (rld-mld/rld %) at the site of stent placement and of any angiographically-detectable lumen narrowing: a lumen reduction >50% was taken as cut-off value for the diagnosis of significant coronary stenosis. Patients with in-stent restenosis, or newly developed stenosis on any branch of the left or in the right coronary artery were excluded from further endothelial function testing.

Acetylcholine-mediated endothelial function testing. In the remaining patients a radiological projection was chosen in order to visualize the proximal and distal part both of the stented and the contra-lateral vessel of the left coronary artery, and an angiogram was repeated as baseline reference. Acetylcholine was then injected in three increasing dosages (10^{-8}, 10^{-7}, 10^{-6} Molar; 3 minutes each), under continuous ECG and blood pressure monitoring. Angiograms were acquired within 1 minute after each infusion. Finally, a bolus (500 µg) of nitro-glycerine (NTG) was administered and the angiogram was repeated. Images were digitally stored for post-processing analysis.

Quantitative coronary analysis. Off-line quantitative coronary analysis was performed by an independent observer (SHJM) with a high volume experience (12-14) using the same system as above. End-diastolic frames were chosen from each angiographic sequences and mean lumen diameter was calculated at baseline, after the maximal acetylcholine dose achieved, and after nitro-glycerine, both for the proximal and the distal part, in the stented and the contra-lateral vessel. Coronary segments selected for analysis had to be straight, devoid of side branches, long at least 1 cm, and, in the case of the stented vessel, far at least 1 cm from the edges of
the stent. Given the range of vessel diameters explored, and being the inter- and intra-observer variability of the computerized system approximately $10^{-1}$ mm, diameter changes of $<5\%$ were considered not relevant (15).

**Statistical analysis.** Descriptive statistics are reported as Mean ± standard deviation (SD), and percentages, respectively for continuous and dichotomous variables. Absolute mean diameter values at baseline, after acetylcholine and nitro-glycerine, were compared by means of a General Linear Model for repeated measures, separately for proximal and distal coronary segments, in stented and contra-lateral vessels. Linear regression analysis between stented and contra-lateral vessels was performed for diameter changes (%) vs. baseline after acetylcholine and nitro-glycerine. To determine the predictive factors for diameters changes (%) vs. baseline, in stented and contra-lateral vessels, we performed univariate regression analysis including clinical and procedural features, and ergometric pre-catheterisation results. Variables with a p value less than 0.40 were incorporated in a multivariable regression analysis to derive a model with statistically significant factors. All p values were two-sided. Only p values less than 0.05 were considered to be significant. Statistical analysis was performed using SAS software, version 6.12 for Windows (SAS, Cary, NC - USA).

**RESULTS**

From January 1998 to July 1998, 37 patients fulfilled the inclusion criteria and gave their permission to perform redo angiography including acetylcholine testing 6 months after inclusion. In two patients no redo angiography was performed due to stent thrombosis within 48 hours after initial therapy. From the remaining patients, acetylcholine testing at 6 months follow-up was not performed in 5 patients due to severe restenosis (2) or logistic reasons (3) respectively, and they were excluded from further analysis. General and procedural characteristics of the 30 patients representing the study population (21 males; mean age: 59 ± 8 years) are reported in Table 1. Among them, no patient demonstrated major inducible ischemia at pre-test bicycle ergometry. However, while 17 patients showed no sign of ischemia at all, in 13 patients one or more minor signs of inducible ischemia were evident (typical angina without significant ECG changes (5); either ST down sloping $<0.1$ mV or T-waves inversion, with or without atypical chest pain (9); and a-symptomatic frequent ventricular ectopic activity (1)).
<table>
<thead>
<tr>
<th>Patients</th>
<th>(n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n)</td>
<td>21 (70 %)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 8</td>
</tr>
<tr>
<td>Coronary Risk Factors:</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>3 (10 %)</td>
</tr>
<tr>
<td>Smoking habit (n)</td>
<td>7 (23 %)</td>
</tr>
<tr>
<td>Hypercholesterolemia (n)</td>
<td>10 (33 %)</td>
</tr>
<tr>
<td>Stented vessel:</td>
<td></td>
</tr>
<tr>
<td>LAD (n)</td>
<td>14 (47 %)</td>
</tr>
<tr>
<td>D/AL (n)</td>
<td>6 (20 %)</td>
</tr>
<tr>
<td>CX (n)</td>
<td>6 (20 %)</td>
</tr>
<tr>
<td>MO/LPL (n)</td>
<td>4 (13 %)</td>
</tr>
<tr>
<td>Therapy with:</td>
<td></td>
</tr>
<tr>
<td>Statins (n)</td>
<td>9 (30 %)</td>
</tr>
<tr>
<td>ACE-inhibitors (n)</td>
<td>6 (20 %)</td>
</tr>
<tr>
<td>Ischemia at follow-up (n)</td>
<td>13 (43 %)</td>
</tr>
<tr>
<td>In-stent lumen reduction (%)</td>
<td>17 ± 9</td>
</tr>
</tbody>
</table>

Table 1: Patient characteristics (n; %): LAD: left anterior descending artery; D/AL: diagonal/anterolateral branch; CX: circumflex artery; MO/LPL: obtuse margin/left posterolateral branch

**Acetylcholine provocative test.** Acetylcholine test was successfully completed in all but 6 patients. In these 6 patients acetylcholine infusion was interrupted because of severe chest pain with or without ECG changes (14) after $10^{-8}$ and $10^{-7}$ Molar (2 and 4 patients respectively). In that case final angiograms were promptly acquired and nitro-glycerine was administered with rapid restoration of the basal conditions. At off-line quantitative analysis, the proximal segment of the stented coronary artery was not evaluated in 6 patients (four out of six had an interrupted acetylcholine test; see above), given the very proximal position of the stent. Moreover, because of vessel overlapping, tortuous course or inadequate filling, three contra-lateral vessels (proximal and distal segments) and one distal segment in another patient could not be evaluated (all with an interrupted test). Basal mean diameters for proximal and distal segments, in the stented and in the contra-lateral vessel, were $3.5 ± 0.6$, $2.7 ± 0.5$, $3.4 ± 0.5$, and $2.6 ± 0.5$ mm, respectively. The proximal coronary segments, both in the stented and in the contra-lateral vessel, did not show any significant change, after acetylcholine and after nitro-glycerine (Figure 1).
Figure 1: Mean diameters (±SD) of proximal (black) and distal (white) coronary segments in stented (♦ n=24; ◊ n=30) and contra-lateral (● n=27; □ n=26) vessels at six months follow-up, as assessed by quantitative coronary angiography. Values are reported at baseline (BL), after acetylcholine (AC) and nitro-glycerine (NTG). * indicates p<0.01 vs. baseline; # indicates p<0.01 vs. acetylcholine.

On the other hand, the distal coronary segments showed a significant vasoconstriction after acetylcholine, both in the stented (2.4 ± 0.6 mm; -11 ± 7 % vs. baseline; p<0.01) and in the contra-lateral vessel (2.3 ± 0.6 mm; -11 ± 6%; p<0.01). Nitro-glycerine administration induced vasodilatation with restoration of basal diameters in the stented (2.8 ± 0.5 mm; +17 ± 12 %; p<0.01 vs. acetylcholine) and in the contra-lateral vessel (2.6 ± 0.6 mm; +13 ± 9 %; p<0.01 vs. acetylcholine) (Figure 1). The vasoconstrictive response (% from baseline) of the distal segments of stented and contra-lateral vessels in the same patient were significantly correlated (R= 0.48; p<0.05) (Figure 2).
At univariate analysis, the occurrence of inducible ischemia was the only predictive factor for vasoconstriction in the distal segment (n=30) of the stented vessel (p<0.01). The odds ratio could not be calculated because all patients without a constrictive response to acetylcholine had a normal stress test (6 out of 17 patients), whereas all patients with inducible ischemia had a more or less constrictive response to acetylcholine (13 patients). No other parameter reported in Table 1 showed statistical significance nor reached the cut-off value (p<0.40) to be incorporated in a multivariable analysis. On the other hand, no parameter was found predictive for distal vasoconstriction in the contra-lateral vessel (n=26). Inducible ischemia (p=0.06) and smoking (p=0.38) showed a tendency toward statistical significance.

While patients with signs of ischemia at pre-catheterisation ergometric test
showed a significantly higher vasoconstrictive response to acetylcholine than controls in the distal segments of the stented coronary artery (-16 ± 7 % vs. -7 ± 6 %; p<0.01), they showed only a tendency toward increased vasoconstriction in the distal segments of the contra-lateral vessel (-14 ± 7% vs. –9 ± 6%; p=0.06) (Figure 3).

Figure 3: Vasoconstriction (%) after acetylcholine in distal segments of stented and contra-lateral vessels, according to the occurrence (black columns) or the absence (white columns) of inducible ischemia at ergometric test. * indicates p<0.01 ischemic vs. controls.

**DISCUSSION**

The main findings of this study are: a) a distal vasoconstrictive response to acetylcholine in non-stenotic stented and contra-lateral vessels, six months after successful coronary stenting; and b) a correlation between inducible ischemia and distal impaired endothelium-dependent vasomotor function in stented but not in contra-lateral non-treated vessels.

Paradoxical vasoconstriction to acetylcholine in the distal epicardial
coronary segments of our study cohort can be reasonably explained by the pre-existence of diffuse atherosclerosis. In fact, as already demonstrated by other authors (16;17), angiographically normal coronary arteries can show pathological vasomotor responses, both in patients with proven atherosclerosis and in patients with only strong risk factors, due to the occurrence of endothelial dysfunction, i.e. unable to counteract the direct vasoconstrictive action of acetylcholine. As confirmed in the present study, blunted endothelium-dependent vasomotor function is homogeneously distributed both in contra-lateral non-stenotic, and in reperfused non-stenotic coronary vessels, thus suggesting that aberrant vasomotion is an independent event from the presence of a flow-limiting stenosis.

Differences in terms of vasomotor function between proximal and distal segments have been previously described after PCI (18;19). Mandinov et al. (19) suggested that changes in coronary flow, distending pressure, or both, could induce an aberrant vasoconstrictive response immediately after reperfusion. El-Tamimi et al. (9) demonstrated the presence of sustained endothelium-dependent vasomotion abnormalities still eight days after a successful coronary angioplasty. The prolonged release from the disrupted plaque of vasoactive substances and inflammatory mediators was suggested to be the reason for these findings (20-23). However, the present study demonstrated a blunted endothelium-dependent vasomotor function homogeneously distributed both in contra-lateral non-stenotic, and in reperfused non-stenotic coronary vessels, six months after the angioplasty procedure, thus suggesting that aberrant vasomotion is an independent event from the presence of coronary stent implantation.

Though stents have proved to be superior to only-balloon angioplasty in terms of acute and long term success (1;2), nevertheless they exert a strong mechanical injury, and require a long period before complete re-endothelisation (24). Stents are also indicated to trigger a diffuse inflammatory process in the coronary artery (25). Our results confirm the findings of Caramori et al. (10), who showed a sustained impairment of endothelium-dependent vasomotor function in distal coronary segments after coronary stenting. They also proved that coronary stenting induces endothelial dysfunction at a higher extent than balloon angioplasty and directional atherectomy. However, they could not demonstrate endothelial dysfunction in the contra-lateral vessel.

As distal coronary segments mirror the influence of vasoactive stimuli on the tributary microvascular circulation (26), vasomotion abnormalities in the epicardial segments may be associated with the presence of microvascular diffuse endothelial dysfunction. According to this hypothesis, in patients
with suspected early atherosclerosis, Zeiher et al. (27) proved a strong correlation between an aberrant vasoconstriction to acetylcholine in distal epicardial vessels and an increase in vascular resistances in the microvascular district. Pre-existing endothelial dysfunction both in the epicardial and the microvascular system, possibly worsened by stent implantation, can make reperfused districts more sensitive to circulating catecholamines (28) or to sympathetic stimuli (29), and lead to myocardial ischemia. Endothelial dysfunction in resistance vessels has been already described as a cause of ischemia in patients with angiographically normal coronary arteries (30). Both a reduction in distal epicardial vasodilative reserve (31) and microvascular spasms (32) claim a role in the pathophysiological mechanism causing ischemia in patients without significant coronary lesions. Though the impairment in endothelium-dependent vasomotor function also found in the contra-lateral vessel might give its contribution, in our study population a clear correlation was found only between inducible ischemia and the presence of marked vasoconstriction in the stented vessel. Thus, for the first time, our study demonstrates that exercise-induced ischemia after successful PCI is related to distal coronary endothelial dysfunction in absence of a significant angiographic stenosis.

**Clinical implications.** Coronary revascularisation (particularly with stents) can be an effective treatment for epicardial coronary stenosis, but it seems inadequate in reverting, when not responsible for worsening, the functional and metabolic abnormalities underlying coronary artery disease. Though in our highly-selected low-risk population no significant correlation was found, medical treatment such as Statins and Ace-inhibitors have proven to reduce ischemic events in patients with coronary atherosclerosis, mainly by restoring endothelial functionality, and may be proposed as a helpful complement for hindering myocardial ischemia after successful coronary stenting (33-35).

**Limitations of the study.** Though untreated contra-lateral vessels can provide an effective in-patient control arm (36), endothelium-dependent vasomotor function was not assessed prior to revascularisation, so that it has not been possible to assess the degree of endothelial dysfunction pre-existing to coronary stenting. However, we expected that the presence of a flow-limiting stenosis would have compromised the correct angiographic assessment of acetylcholine-induced vasomotion. Moreover, though ECG changes have been reported as a reliable index for endothelium-related inducible ischemia (37), no myocardial perfusion test (scintigraphy or PET scan) was performed, so the actual impairment in myocardial perfusion due to distal vasoconstriction could not be quantified.
CHAPTER 9

CONCLUSIONS

Endothelium-dependent vasomotor function both in reperfused and non-stenotic contra-lateral vessels appears impaired, six months after successful coronary stenting. Marked endothelial dysfunction in the epicardial vessels submitted to coronary stenting, and possibly in the tributary microcirculation, can be the cause for mild inducible ischemia at late follow-up.

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