Rapid Communication

Endothelial function in patients with slow coronary flow and normal coronary angiography

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INTRODUCTION

Atherosclerotic heart disease usually manifests as angina and is diagnosed by stress imaging tests and coronary angiography (1), but some patients with typical angina and documented myocardial ischemia have normal coronary arteries (2), a clinical picture called cardiac syndrome X (3). Endothelial (4) and microvascular (5) dysfunction have been suggested to play a pathogenic role in this situation. Patients with slow coronary flow (SCF) (6) and endothelial dysfunction (7) are both at increased risk for cardiovascular events. Several methods to measure endothelial injury can provide clinical opportunities to identify these patients (8), but the evaluation of endothelial function in arterial and venous vascular beds has not yet been performed. The aim of this study was to evaluate the arterial and venous endothelial functions in patients with stable angina and normal coronary anatomy but SCF on a cardiac angiogram.

MATERIALS AND METHODS

This case-control study was previously approved by the institutional ethics committee and involved 21 patients referred for coronary angiography to evaluate coronary artery disease. The inclusion criteria were angina, a myocardial perfusion defect (stress imaging test) and normal coronaries (coronary angiography). The exclusion criteria were myocardial infarction in the last 60 days, heart transplantation, a revascularization procedure, left ventricular dysfunction, systemic inflammatory diseases, autoimmune diseases, obesity, smoking, hematological disorders, hemodialysis and the current use of anticoagulant, corticosteroid or immunosuppressive therapy.

The patients underwent elective coronary angiography using the standard Judkins technique (9). The coronary arteries were visualized in the left and right oblique planes using cranial and caudal angles. Left ventriculography was performed in the right anterior oblique view. The injection of contrast medium was recorded at a speed of 30 frames/s. Coronary flow was quantified objectively by two independent observers, who were blinded to the clinical characteristics of the participants. The TIMI frame count was assessed as described in the literature, and the longer left anterior descending artery frame counts were corrected by dividing by 1.7 to derive the corrected TIMI frame count (CTFC). The patients with a CTFC greater than two standard deviations from the normal published range for the particular vessel were accepted as having SCF (10). The average TIMI frame count for each subject was calculated by adding the TIMI frame counts for the left anterior descending artery, left circumflex artery, and right coronary artery and then dividing the value obtained by three.

The participants had their blood collected in the fasting state to measure glycemia, total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides (automated enzymatic commercial kits; Roche, Mannheim, Germany), insulin (enzyme immunoassay commercial kits; Abbot-Murex, Park, IL, USA), and C-reactive protein (nephelometry, nephelometer BN100, Dade Behring Inc., Marburg, Germany). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described (11). The patients were instructed not to use medications for 72 h before the endothelial evaluations.

Venous endothelial function was evaluated by the dorsal hand vein technique (12,13). A 23-gauge butterfly needle was inserted into a vein on the back of the hand. A continuous infusion (Harvard infusion pump, South Natick, MA) of saline solution (rate, 0.3 mL/min) was started. A tripod holding a linear variable differential transformer (model 025 MHR, Shaevitz Engineering, Pennsauken, NJ) was mounted on the hand to measure the diameter of the vein. The readings were taken at a congestive pressure of 40 mmHg by the inflation of a blood pressure cuff placed on the upper portion of the arm under study. The diameter of the vein during the saline infusion with the cuff inflated was defined as 100% relaxation. The vein was preconstricted by infusing increasing doses (7 min each) of phenylephrine (37-25000 ng/mL) until the dose that produced approximately 70% constriction (ED70%) of the vein was identified; this dose was used as a reference for the subsequent experiments. The vasodilation produced by six doses (12-12000 ng/mL) of

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No potential conflict of interest was reported.
acetylcholine (endothelium-dependent) and three doses (156-3125 ng/mL) of sodium nitroprusside (endothelium-independent) was analyzed (3 min each). The individual effects were analyzed as the percentage of maximum venodilation (13).

Flow-mediated vasodilation (FMD) was measured to evaluate the arterial endothelium-dependent vasodilation using a high-resolution vascular ultrasound (EnVisor CHD; Philips, Bothell, WA, USA) and a 3-12 MHz linear-array transducer (L12-3, Philips, Bothell, WA, USA). Briefly, the change in the brachial artery diameter after 60 s of reactive hyperemia was compared with a baseline measurement after the deflation of a cuff that had been placed around the forearm and inflated to 50 mmHg above the systolic blood pressure for 5 min (14). The diameter increase after a sublingual nitroglycerin spray (0.4 mg) was used as a measurement of endothelium-independent vasodilation. The vessel diameter responses to reactive hyperemia and to nitroglycerin were expressed as the percentage changes relative to the diameter immediately before drug administration.

The data are presented as the means ± SD. The distributions of the variables were determined using Shapiro–Wilk tests of normality. GraphPad Software Prism 4.0 was used for comparisons with ANOVA (using the Bonferroni post hoc test), Fisher's exact test, the Mann-Whitney test and the t-test for comparisons with normal coronary flow; their characteristics are presented in Table 1. The SCF group had a higher body mass index, age, systolic blood pressure for 5 min (14). The diameter increase after a sublingual nitroglycerin spray (0.4 mg) was used as a measurement of endothelium-independent vasodilation. The vessel diameter responses to reactive hyperemia and to nitroglycerin were expressed as the percentage changes relative to the diameter immediately before drug administration.

Here, we show that patients with SCF, normal coronary arteries and documented myocardial ischemia display arterial and venous endothelial dysfunction, which was not observed in similar patients with normal coronary flow.

DISCUSSION

Here, we show that patients with SCF, normal coronary arteries and documented myocardial ischemia display arterial and venous endothelial dysfunction, which was not observed in similar patients with normal coronary flow. In addition, we show that venous endothelial dysfunction is observed in patients with SCF and normal coronary arteries. Multiple abnormalities have been reported to explain cardiac syndrome X, including endothelial dysfunction (15), increased oxidative stress (16) and vascular inflammation (17). The observation that SCF has also been associated with metabolic abnormalities (18) and endothelial dysfunction (19) may link these abnormalities. Previous studies showed arterial endothelial dysfunction in patients with SCF (15,17,20). One of these studies reported that CTFC correlates with endothelial function, even in individuals with normal coronary flow (15). However, both the arterial and venous endothelium can be injured in patients with cardiovascular risk factors (21,22), and their treatment can reverse the endothelial dysfunction in both vascular beds (22), which could occur in patients with SCF. Under physiological conditions, the venous endothelium is subjected to a lower shear stress and O2 concentration compared with the arterial endothelium (23), but arterial vasodilation is reduced when the venous endothelium is injured (24),

Table 1 - Baseline clinical and laboratory characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 12)</th>
<th>Slow Coronary Flow (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M)</td>
<td>4/8</td>
<td>3/6</td>
<td>0.999</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.2 ± 10</td>
<td>58.0 ± 10</td>
<td>0.230</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.4 ± 3</td>
<td>29.9 ± 5</td>
<td>0.044</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.8 ± 10</td>
<td>133.3 ± 18</td>
<td>0.116</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.1 ± 9</td>
<td>82.2 ± 9</td>
<td>0.343</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.8 ± 1</td>
<td>14.7 ± 1</td>
<td>0.608</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.29 ± 0.2</td>
<td>0.48 ± 0.3</td>
<td>0.145</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>198.7 ± 36</td>
<td>183.2 ± 55</td>
<td>0.475</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>88.9 ± 26</td>
<td>131.1 ± 52</td>
<td>0.047</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>121.4 ± 30</td>
<td>113.5 ± 54</td>
<td>0.703</td>
</tr>
<tr>
<td>HDL-c (mg/dL)</td>
<td>59.5 ± 14</td>
<td>43.4 ± 8</td>
<td>0.006</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>86.6 ± 8</td>
<td>99.2 ± 10</td>
<td>0.019</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>5.4 ± 3</td>
<td>10.3 ± 6</td>
<td>0.051</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 ± 1</td>
<td>4.7 ± 3</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Data are shown as the means ± SD. HOMA-IR = homeostasis model assessment of insulin resistance.
suggesting that substances produced by the venous endothelium can influence arteriolar tonus (24,25). Thus, venous endothelial dysfunction can contribute to the microvascular spasm observed in patients with SCF.

Endothelial dysfunction and increased reactive oxygen species may trigger the production of cytokines and cell adhesion molecules (26). Patients with SCF present decreased plasma concentrations of adiponectin (27) and increased serum levels of soluble adhesion molecules (ICAM-1, VCAM-1 and E-selectin) (19) and C-reactive protein (28). Our data revealed that C-reactive protein was associated with arterial endothelial dysfunction, suggesting additional repercussions of systemic inflammation in the arterial bed.

Lipid profile changes, insulin resistance and the frequency of metabolic syndrome were more frequent in patients with SCF and endothelial dysfunction, as demonstrated in the literature (19,29), suggesting a common ground for these alterations (18).

Our study has several limitations: the sample size was small, it was a single-center study and only patients with stable conditions were selected. The observational nature of the study does not allow cause and effect relationships to be determined. Nonetheless, the completeness of the evaluations performed in the study does not allow cause and effect relationships to be determined. Nevertheless, the completeness of the evaluation is a strength that can provide new insights into this puzzling phenomenon.

In conclusion, patients with SCF and normal coronary arteriograms venous and arterial endothelial dysfunction, suggesting that this condition might be a systemic vascular phenomenon.

ACKNOWLEDGMENTS
The authors would like to thank CNPq, CAPES, and FAPERGS.

AUTHOR CONTRIBUTIONS
Signori LL, Quadros AS, Shruzz G, Dipp T, Lopes RD and Schaan BD conceived and designed the study and were responsible for the manuscript draft, data analysis and interpretation, critical revision for the important intellectual content of the manuscript and approval of the final version of the manuscript.

REFERENCES
22. Sezgin AT, Sigirci A, Barutcu I, Topal E, Sezgin N, Ozdemir R, et al. Lipid profile changes, insulin resistance and the frequency of metabolic syndrome were more frequent in patients with SCF and endothelial dysfunction, as demonstrated in the literature (19,29), suggesting a common ground for these alterations (18).
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24. In conclusion, patients with SCF and normal coronary arteries present venous and arterial endothelial dysfunction, suggesting that this condition might be a systemic vascular phenomenon.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 12)</th>
<th>Slow Coronary Flow (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venoconstriction (% phenylephrine)</td>
<td>72.4 ± 5</td>
<td>73.6 ± 10</td>
<td>0.744</td>
</tr>
<tr>
<td>Emax (%, acetylcholine)</td>
<td>87.2 ± 34</td>
<td>52.7 ± 27</td>
<td>0.019</td>
</tr>
<tr>
<td>Drug concentration</td>
<td>142.7 ± 39</td>
<td>139.9 ± 35</td>
<td>0.440</td>
</tr>
<tr>
<td>ED50 (%, phenylephrine)</td>
<td>175 ± 167</td>
<td>452 ± 788</td>
<td>0.560</td>
</tr>
<tr>
<td>Emax (ng/mL acetylcholine)</td>
<td>4500 ± 4031</td>
<td>2948 ± 3834</td>
<td>0.196</td>
</tr>
<tr>
<td>Emax (ng/mL sodium nitroprusside)</td>
<td>1496 ± 619</td>
<td>1822 ± 781</td>
<td>0.237</td>
</tr>
<tr>
<td>Flow-mediated vasodilation (%)</td>
<td>13.3 ± 5</td>
<td>7.5 ± 5</td>
<td>0.022</td>
</tr>
<tr>
<td>Nitroglycerin-induced vasodilation</td>
<td>18.8 ± 6</td>
<td>13.4 ± 7</td>
<td>0.104</td>
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</tbody>
</table>

Data are shown as the means ± SD. ED50 = percentage of venuconstriction; Emax, = maximum effect.
Endothelial function in slow coronary flow

Signori et al.


