TRANSLATIONAL PHYSIOLOGY

Endothelial dysfunction occurs in peripheral circulation in patients with acute and stable coronary artery disease

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Thanayasiri, Panurath, David S. Celermajer, and Mark R. Adams. Endothelial dysfunction occurs in peripheral circulation in patients with acute and stable coronary artery disease. Am J Physiol Heart Circ Physiol 289: H513–H517, 2005; doi:10.1152/ajpheart.01086.2004.—Atherosclerosis is a diffuse, systemic process. In addition, acute coronary syndromes (ACS) are associated with inflammatory marker elevations that are hypothesized to affect the function of nonculprit coronary as well as peripheral vessels. We investigated whether femoral vascular reactivity and/or fibrinolytic capacity are impaired in ACS patients over and above any dysfunction associated with stable coronary artery disease. Patients undergoing diagnostic coronary angiography \(n = 42\) total, 14 patients/group) were recruited into three groups as follows: 1) stable coronary syndromes (SAP group), 2) ACS as defined by rest angina with ECG changes and troponin rise (ACS group), and 3) angiographically normal coronary arteries (control group). After diagnostic coronary angiography, femoral artery endothelial and smooth muscle function were assessed by infusing acetylcholine (ACh) and nitroglycerin (GTN), and tissue-type plasminogen activator (t-PA) release across the femoral circulation was measured as the difference between arterial and venous concentrations before and after ACh and GTN stimulation. There were no significant differences between groups in relevant baseline characteristics apart from significantly higher C-reactive protein levels and reduced net t-PA release in the ACS group at baseline \((P < 0.05)\). The ACS and SAP groups had equivalent angiographic severity of coronary artery disease. Endothelial-dependent dilatation was significantly higher in control individuals \((14.9 \pm 9.1\% \text{ vs } 4.1 \%, P < 0.001)\) compared with either stable patients \((2.3 \pm 8.1\% \text{ vs } 1.4 \%, P = 0.2)\) or those with unstable syndromes \((2.6 \pm 8.9\% \text{ vs } 1.4 \%, P = 0.2)\). Although baseline t-PA release was impaired in the ACS patients \((0.09 \pm 0.06 \text{ compared with } 0.39 \pm 0.33 \text{ and } 0.49 \pm 0.56 \text{ ng/ml; } P = 0.03)\), stimulation of t-PA release by ACh and GTN occurred only in the control subjects and not in the ACS or SAP patients. Coronary artery disease is associated with impaired endothelium-dependent dilatation and impaired stimulation of t-PA release in the systemic circulation. These aspects of endothelial dysfunction, however, were equally severe in acute and chronic coronary syndrome patients.

tissue-type plasminogen activator; nitroglycerin; acetylcholine; cardiac inflammation; thrombosis; vasodilation

ACUTE CORONARY SYNDROMES OCCUR when coronary atherosclerotic plaques become unstable with lesion rupture and/or subsequent thrombosis (12). In recent years, it has become apparent that this process is largely an inflammatory one that involves the interaction of leukocytes, cytokines, and endothelium (13). It has been hypothesized that this inflammatory activation with systemic cytokine release may lead to distant endothelial dysfunction in nonculprit coronary as well as peripheral arteries (16, 24, 29, 30). However, whether systemic endothelium-dependent dilatation and/or production of tissue-type plasminogen activator (t-PA) are altered in the setting of acute coronary syndromes over and above those associated with stable coronary artery disease has not been studied.

Arterial endothelium normally controls a number of important functions vital to vascular health including vasodilation (11) and thromboresistance (4, 10, 27), which are related, respectively (in part), to nitric oxide (NO) and t-PA release. We hypothesized that the systemic process seen in acute coronary syndromes would extend to the peripheral circulation with reduced endothelium-dependent dilatation and t-PA release.

METHODS

Subjects. Three groups of patients presenting to the cardiac catheterization laboratory of the Royal Prince Alfred Hospital were recruited as follows: 1) those with stable coronary syndromes as defined by no resting angina and no change in their symptom pattern for the preceding 6 mo (SAP group), 2) patients with an acute coronary syndrome as defined by resting angina with ECG changes and an increase in troponin (ACS group), and 3) patients shown to have angiographically normal coronary arteries (control group). Studies were performed only after the withdrawal of nitrate drugs, and the three subject groups were matched for age, gender, smoking status, presence of diabetes, and, in the case of the two groups with coronary artery disease, severity of coronary artery disease, which was assessed using the modified Gensini score described in detail elsewhere (2). Briefly, the most severe stenosis in each of eight coronary segments was graded from 1 to 4 \((\text{grade } 1, 1–49\% \text{ lumen diameter reduction; } \text{grade } 2, 50–74\% \text{ stenosis; } \text{grade } 3, 75–99\% \text{ stenosis; and } \text{grade } 4, 100\% \text{ occlusion})\) to yield a total score between 0 and 32, which provides a measure of both severity and extent. Apart from control subjects, all patients were taking aspirin \((150 \text{ mg daily})\), and none were treated with other antiplatelet agents before the study. All studies were performed after administration of 2,500 units of heparin, which has been shown not to affect maximal t-PA release (23). Patients were excluded who were <18 yr of age, pregnant, or had S-T elevation myocardial infarction, decompensated congestive heart failure, life-threatening arrhythmias, uncontrolled hypertension (systolic blood pressure >170 mmHg despite therapy), or peripheral vascular disease with symptomatic claudication, absent lower limb pulses, or angiographic iliofemoral stenoses >40%. Patients taking vasoactive medications including nitrates of any kind, angiotensin

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l-converting enzyme inhibitors, and calcium antagonists were also excluded.

**Study protocol.** Before the procedure, a full history and physical examination was obtained, and blood was drawn (fasting) for cholesterol analysis, high-sensitivity C-reactive protein measurement (Denka Seiken; Tokyo, Japan), routine biochemistry, and blood count. Diagnostic cardiac catheterization was completed in the usual manner using a 6-Fr sheath in the femoral artery and a 5-Fr sheath in the femoral vein. After coronary angiography but before any planned percutaneous intervention, the study protocol was carried out as outlined below. The arterial sheath was connected to pressure-monitoring equipment via a three-way tap with an infusion pump connected to deliver intra-arterial infusions. All intra-arterial infusions were delivered in 5% dextrose at 0.8 ml/min for 3 min; there was a 10-min washout period between the acetylcholine (ACh) infusions and nitroglycerin but no interval between increasing doses of ACh.

The infusions consisted of dextrose alone (control), ACh at three doses (with estimated concentrations of $10^{-5}$, $10^{-4}$, and $10^{-3}$ M; acetylcholine chloride; Novartis Australia), dextrose alone (recontrol), and nitroglycerin (100 μg/ml; glyceryl trinitrate; David Bull). ACh doses were calculated assuming a resting femoral artery flow of 400 ml/min with doses of ACh from 0.001 to 0.1 mg/ml, although with increase in blood flow at higher doses, the actual concentrations may have been slightly lower. Each infusion was followed by an injection of 9 ml of iodine contrast material while 4-s cine angiograms were acquired.

Throughout each study, blood pressure, heart rate, ECG, and clinical status were closely monitored. The tube and table heights and anteroposterior and lateral angles of the image intensifier were recorded and kept constant for each of the research cine angiograms.

Levels of t-PA were measured after the baseline control infusion, after the highest dose of ACh, and after the administration of nitroglycerin. Blood was taken simultaneously from both the arterial sheath and the venous sheath onto ice and was centrifuged immediately at 3,000 rpm for 15 min at 4°C; plasma was then collected and stored at −80°C until analysis. Samples were assayed by a blinded independent observer using a commercially available kit for enzyme-linked immunosorbent assay of t-PA (Coaliza t-PA; Chromogenix, Germany). This software is used to search densities with edge-seeking algorithms for each segment for each infusion. The standard deviation of the diameters from these frames was used to obtain a pooled standard deviation for each segment and infusion.

Blood flow volume was estimated using the method described by Kinlay and colleagues (9) adapted for use in the peripheral circulation. Average blood flow velocity was measured using the thrombolysis in myocardial infarction (TIMI) frame-count method of contrast along a known length of the femoral artery filmed at 15 frames/s; blood flow velocity (in mm/s) = [(15 × length)/frame count]. Blood flow volume was then calculated using the average cross-sectional area, which was derived from the average diameter of the length assessed by quantitative coronary angiography as follows: average cross-sectional area = π (average radius$^2$) of the femoral artery, multiplied by the blood flow velocity (expressed in ml/min).

**Statistical methods.** Responses to ACh are presented as the continuous variable of percent dilatation or constriction compared with baseline diameter. The primary end point was predefined as the difference in response to the maximal dose of ACh between the three groups; other predefined end points were diameter changes in response to other doses of ACh and nitroglycerin as well as changes in blood flow and differences in baseline and stimulated t-PA release. A one-way ANOVA was used to compare the three groups and was followed by a post hoc Scheffé’s test to examine differences between groups. Paired t-tests were used to examine differences within groups (for example, changes in t-PA in the control group after stimulation compared with baseline). Results are displayed as means ± SD, and statistical significance was determined at the $P < 0.05$ level. Sample-size calculation was based on earlier studies on the coronary arteries. In these studies, ACh-induced constriction of the coronary artery in patients with coronary artery disease typically ranged from +10 to −30% with a SD of 15%. In the femoral arteries, however, based on an initial group of 10 patients studied, we expected a smaller range of vasomotor responses of +15 to −10% with a SD of 10%. We expected to see a difference in the means of this continuous exposure variable of 25%. This yields a sample size of 12 per group for a power of 80% to detect this difference at the 0.05 significance level. This protocol was approved by the local Ethics Review Committee.

**RESULTS**

**Baseline characteristics.** The baseline characteristics of the patients involved in the study are summarized in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Stable Coronary</th>
<th>Acute Coronary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td></td>
<td>58±10</td>
<td>61±9</td>
<td>59±7</td>
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<tr>
<td>Male gender, %</td>
<td></td>
<td>71</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>Nonsmokers, %</td>
<td></td>
<td>79</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td></td>
<td>43*</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td></td>
<td>36*</td>
<td>93</td>
<td>86</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td></td>
<td>5.0±0.9</td>
<td>4.9±1.6</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td></td>
<td>1.6±0.1</td>
<td>1.9±0.7</td>
<td>2.1±2.3</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td></td>
<td>14*</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td></td>
<td>145±33</td>
<td>153±26</td>
<td>148±31</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td></td>
<td>0</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td></td>
<td>5.5±1.6</td>
<td>5.6±0.8</td>
<td>6.2±2.0</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
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<td>5.1±0.9</td>
<td>5.4±1.8</td>
<td>6.1±2.2</td>
</tr>
<tr>
<td>Creatinine, mmol/l</td>
<td></td>
<td>76±16</td>
<td>80±15</td>
<td>75±24</td>
</tr>
<tr>
<td>Troponin T level, μg/l</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1.4±1.0*</td>
</tr>
<tr>
<td>Gensini score</td>
<td></td>
<td>0*</td>
<td>9.1±3.2</td>
<td>8.7±4.2</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td></td>
<td>2.4±1.7</td>
<td>2.4±1.9</td>
<td>16.3±21.8*</td>
</tr>
<tr>
<td>Femoral artery diameter, mm</td>
<td></td>
<td>4.3±1.1</td>
<td>4.9±1.5</td>
<td>4.8±1.1</td>
</tr>
</tbody>
</table>

Values shown as ranges are means ± SD; $n = 14$ individuals/group (total, 42 individuals). *$P < 0.05$ for comparison between outcomes.
Subjects in the three groups were well matched for age and gender, although the control group had a lower incidence of some risk factors such as hypercholesterolemia and hypertension (as expected). Also, control patients were receiving less treatment with cholesterol-lowering medications. Patients with acute and chronic coronary syndromes had similar severity of coronary artery disease. There was a significantly higher incidence of troponin elevation in those patients with an acute coronary syndrome as per our definition of this condition, and these patients also had an elevated C-reactive protein level compared with stable patients and control individuals (16.3 ± 21.8 compared with 2.4 ± 1.9 and 2.4 ± 1.7 mg/ml; P < 0.05). By definition, control patients had a Gensini score of 0; however, the scores were similar in acute and chronic coronary syndromes at 8.7 ± 4.2 and 9.1 ± 3.2. In both of these groups, there was a nonsignificant relationship between total cholesterol level and Gensini score.

**Femoral vascular reactivity studies.** There was a significant impairment in large arterial endothelium-dependent dilatation in response to ACh in both stable and acute coronary syndrome patients compared with control patients, whereas there was no significant difference in the response to nitroglycerin between groups (Fig. 1). Control patients exhibited a dose-dependent dilatation response of the femoral artery with increasing doses of ACh; however, the other two groups had no significant dilator response at any dose of ACh. Regarding the microcirculation, there was also a significant difference in blood flow at maximal ACh dose with control patients demonstrating an increased flow response to ACh and those patients with stable or acute disease showing no change (Fig. 2).

On univariate analysis, there was a significant positive correlation between the endothelium-dependent dilatation response and use of cholesterol-lowering therapy (r = 0.32; P = 0.04) and normal coronary arteries (r = 0.35; P = 0.02); there were also significant negative correlations between the endothelium-dependent dilatation response and resting vessel diameter (r = -0.84; P = 0.001), C-reactive protein level (r = -0.32; P = 0.03), concentration of t-PA (r = -0.33; P = 0.03), and fasting glucose level (r = -0.47; P = 0.008). On multivariate analysis, the only independent factors related to endothelium-dependent response to ACh were normal coronary arteries (control group) and vessel diameter.

**Fibrinolytic activity studies.** At baseline, the absolute arterial concentration of t-PA was significantly elevated in the group of patients with an acute coronary syndrome (10.6 ± 9.3 compared with 5.4 ± 3.5 and 5.6 ± 3.2 ng/ml; P = 0.04). However, net levels of t-PA release were significantly lower in the group of patients with an acute coronary syndrome compared with those with stable coronary syndromes and control patients (0.09 ± 0.06 compared with 0.39 ± 0.33 and 0.49 ± 0.56 ng/ml; P = 0.03). After ACh and nitroglycerin infusions, there was a significant increase in net t-PA release in control patients only compared with baseline (P < 0.05). Thus net t-PA release was significantly higher in the control group compared with either the acute or stable coronary syndrome groups, which were similar to one another (Fig. 3).

**DISCUSSION**

Acute coronary syndromes with plaque destabilization and rupture are associated with systemic cytokine release and inflammatory activation (11). It was previously hypothesized (16, 24, 29, 30) that this systemic inflammatory response is associated with widespread endothelial dysfunction that involves the nonculprit coronary arteries as well as peripheral arteries. In this, the first in vivo human study to examine peripheral endothelial function in acute and chronic coronary patients, however, we have documented that acute coronary syndromes were not associated with any additional loss of endothelium-dependent dilatation or stimulated t-PA release over and above that seen in stable coronary artery disease. This finding was consistent in both small and large vessels in the femoral artery distribution and contrasted with the normal conduit vessel dilatation and increased flow seen in subjects without coronary artery disease. Although acute coronary syn-
dromes were associated with a small reduction in baseline fibrinolytic capacity, both stable and acute coronary syndromes demonstrated a lack of stimulated release of t-PA. These findings argue against the concept of a generalized “systemic endotheliopathy” associated with acute coronary syndromes as previously suggested by many (16, 24, 29, 30).

The endothelium performs a wide range of important homeostatic functions; it controls vascular tone, growth, and permeability as well as defensive processes such as control of thrombosis and inflammation during hemostasis and repair (1). The loss of coronary vasodilatation in response to increased flow that occurs in stable coronary artery disease is well documented and has been shown to be associated with loss of normal endothelial function (15). Furthermore, improvement in endothelium-dependent dilatation may be associated with improved coronary flow and reduction of stable symptoms (32). For a number of reasons, there is less information available in the setting of acute coronary syndromes. First, although there are many animal and cellular models of atherosclerosis, there is a paucity of models of plaque destabilization and acute coronary syndromes (16). Second, in the setting of acute coronary syndromes, the safety of studying coronary endothelial function is not acceptable. There are, however, prospective data linking impaired endothelium-dependent dilatation and increased risk of future coronary events. A number of investigators (7, 8, 18, 22, 25, 28, 31) have found an association between loss of endothelium-dependent dilatation and risk of coronary and other vascular events using both coronary and peripheral arteries, and this association has been observed for both conduit and resistance vessels. Although these findings suggest that acute coronary syndromes are associated with a generalized “systemic endotheliopathy,” the studies included relatively small and selected cohorts of patients and subjects were not assessed at the time of an acute coronary syndrome. Although subjects with peripheral endothelial dysfunction appear more likely to develop acute coronary syndromes over subsequent years, it is possible that by the time of clinical presentation, as investigated in our study, peripheral endothelial function is uniformly impaired irrespective of the type of clinical presentation.

In the setting of acute coronary syndromes, there is widespread inflammatory activation that can be detected systemically and to varying extents in nonculprit coronary arteries (5). This inflammatory activation is evident from the elevation of C-reactive protein. C-reactive protein is elevated in acute coronary syndromes and correlates with the risk of future clinical cardiac events in asymptomatic subjects and adverse outcomes following an acute coronary syndrome (14, 17). Recently, it has become apparent that C-reactive protein may contribute directly to plaque destabilization and vascular events via a number of actions; prime among these is C-reactive protein’s effect on NO production (11). C-reactive protein, at concentrations of 25 mg/l in vitro (similar to the concentration of the acute coronary syndrome subjects in this study), is able to powerfully attenuate NO production through a post-transcriptional effect on endothelial NO synthase mRNA stability (33). Despite this, the present in vivo study found no relationship between C-reactive protein and endothelium-dependent dilatation of the femoral artery, and subjects with acute coronary syndromes had no additional impairment of endothelium-dependent dilatation compared with stable subjects despite significantly higher levels of C-reactive protein. These findings suggest that C-reactive protein may not be clinically important in reducing NO availability in the peripheral vessels of patients with advanced coronary artery disease.

Apart from impaired endothelium-dependent dilatation, other aspects of endothelial physiology may be disordered locally and distantly in acute coronary syndromes [for example, reduced fibrinolytic activity (29, 30)]. An important component of the fibrinolytic system, t-PA is released from normal endothelial cells constitutively and may be rapidly augmented in response to a number of stimuli (21). It has only been recently possible to reliably measure the net release of t-PA using commercially available assays; however, recent studies have shown that a number of factors can affect arterial fibrinolytic capacity in humans. Factors such as cigarette smoking, extent of coronary atherosclerosis, and aging have been shown to impair the net release of t-PA, whereas exercise may improve its release (19–21, 26). The control of t-PA release has not been fully elucidated; however, its release is augmented by a number of factors such as bradykinin and substance P and also by some cytokines such as tumor necrosis factor-α (6, 21). In the present study, controls without coronary atherosclerosis exhibited an increase in t-PA release with ACh and nitroglycerin consistent with a positive effect of NO; however, in the setting of coronary atherosclerosis, both stable and acute, this stimulated release was not seen. Whether this is secondary to a loss of NO availability in subjects with coronary atherosclerosis requires further study.

Although this study had a small number of patients and was cross-sectional in nature, the technique used for studying endothelium-dependent dilatation (both large and small vessel) had significant power to detect a difference between the stable and acute groups of patients. In addition, the two coronary disease groups had extremely similar results yet were significantly different from control subjects. Furthermore, the groups

Fig. 3. Tissue-type plasminogen activator (t-PA) release was measured as the difference between arterial and venous concentrations for the three groups of subjects at baseline, at peak dose of ACh (10 \(^{-6}\) M), and after nitroglycerine administration. There was a significantly lower release in those subjects with unstable coronary syndromes compared with controls and those with stable administration. There was a significantly lower release in those subjects with unstable coronary syndromes compared with controls and those with stable angina at baseline (\(* * p < 0.05\)). Control patients demonstrated a significant increase in t-PA release with ACh (10 \(^{-6}\) M) and nitroglycerine administration compared with baseline levels; however, there were no changes in the other two groups after ACh or nitroglycerin infusion.
of coronary artery disease patients were well matched for other factors such as severity of coronary artery disease and presence of risk factors. Although patients in the coronary disease groups were well matched for systemic factors, it is possible that there are pathophysiological factors at the culprit site to explain the results of this study. A number of other measures of endothelial physiology were not assessed such as t-PA activity or plasminogen activator inhibitor-I levels; however, recent studies have demonstrated that these measures correlated well with the factors measured (3, 19).

In conclusion, we found that patients with both stable and acute coronary syndromes have marked endothelial dysfunction as assessed by decreased endothelium-dependent dilatation in conduit and resistance vessels as well as failure of stimulated release of t-PA. Acute coronary syndromes were not associated with a further impairment of these endothelial functions in the systemic circulation, however, over and above that seen in patients with stable disease.

GRANTS

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REFERENCES