Local Production of Soluble Urokinase Plasminogen Activator Receptor and Plasminogen Activator Inhibitor-1 in the Coronary Circulation Is Associated With Coronary Endothelial Dysfunction in Humans

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Background—Soluble urokinase plasminogen activator receptor (suPAR) is a proinflammatory biomarker associated with immune activation and fibrinolysis inhibition. Plasminogen activator inhibitor (PAI-1) is associated with excessive fibrin accumulation, thrombus formation, and atherosclerosis. The relationship between cross-coronary suPAR and PAI-1 production and endothelial dysfunction remains unknown.

Methods and Results—Seventy-nine patients (age 53±10 years, 75% women) with angina and normal coronary arteries or mild coronary artery disease (<40% stenosis) on angiogram underwent acetylcholine assessment of epicardial endothelial dysfunction (mid–left anterior descending coronary artery diameter decrease >20% after acetylcholine) and microvascular endothelial dysfunction (coronary blood flow change <50% after acetylcholine). Simultaneous left main and coronary sinus suPAR and PAI-1 levels were measured in each patient before acetylcholine administration, and cross-coronary suPAR and PAI-1 production rates were calculated. Patients’ characteristics, except for age (51±10 versus 57±9, P=0.02), and resting coronary hemodynamics were not significantly different between patients with (26%) versus without (74%) epicardial endothelial dysfunction. Patients’ characteristics and resting coronary hemodynamics were not significantly different between those with (62%) and those without (38%) microvascular endothelial dysfunction. Patients with microvascular endothelial dysfunction demonstrated local coronary suPAR production versus suPAR extraction in patients with normal microvascular function (median 25.8 [interquartile range 12.1, 23.7] versus 12.7 [52.0, −74.8] ng/min, P=0.03). Patients with epicardial endothelial dysfunction had higher median coronary PAI-1 production rates compared with those with normal epicardial endothelial function (1224.7 [12 940.7, −1915.4] versus 187.4 [4 453.8, 0.03]).

Conclusions—suPAR is released in coronary circulation of patients with microvascular endothelial dysfunction and extracted in those with normal microvascular function. Cross-coronary PAI-1 release is higher in humans with epicardial endothelial dysfunction. (J Am Heart Assoc. 2018;7:e009881. DOI: 10.1161/JAHA.118.009881.)

Key Words: coronary circulation • endothelial dysfunction • epicardial • microvascular dysfunction • plasminogen activator • soluble urokinase plasminogen activator receptor
suPAR, PAI-1, and Coronary Endothelial Dysfunction  Corban et al

Clinical Perspective

What Is New?

- Humans with early atherosclerosis and endothelial dysfunction have higher local cross-coronary soluble urokinase plasminogen activator receptor and plasminogen activator inhibitor-1 production.
- Increased local coronary soluble urokinase plasminogen activator receptor production in the coronary circulation of patients with microvascular endothelial dysfunction implicates immune and fibrinolytic pathways in the development of coronary endothelial dysfunction and increased cardiac events.
- Increased local coronary production of plasminogen activator inhibitor in patients with epicardial endothelial dysfunction suggests that impaired or ineffective local fibrinolysis in response to possible subclinical coronary thrombosis occurs concomitantly with endothelial dysfunction in the development of coronary artery disease.

What Are the Clinical Implications?

- Findings of this study may allow for identification of patients with impaired coronary vasomotion and increased risk of coronary artery disease progression who would benefit from initiation of early aggressive medical management.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request. Data sharing is subject to limitations of the informed consent and Mayo Institutional Review Board approval.

Patient Population

Patients who underwent a clinically indicated coronary angiography for angina symptoms at our institution were screened for inclusion in this study. The study was approved by the Mayo Institutional Review Board, and written informed consent was obtained. The study population consisted of 79 consecutive patients with angina who were found to have angiographically normal coronary arteries or mild epicardial CAD (<40% stenosis). Those patients subsequently underwent coronary vasomotor testing during the coronary catheterization procedure described below. Exclusion criteria included acute coronary syndrome presentation, myocardial infarction, or cerebrovascular accident within the past 6 months, previous percutaneous coronary intervention, use of radiographic contrast agents within 12 hours before catheterization, advanced chronic kidney disease, cardiomyopathy (ejection fraction <45%), active malignancy, local or systemic infectious disease within 4 weeks before catheterization, inflammatory diseases, and pregnancy. All patients fasted for at least 8 hours and withheld all prescription medications that could affect coronary vasoreactivity for at least 48 hours before the study procedure.

Study Protocol

Systemic and Local Coronary Blood Sampling

Routine preprocedural laboratory values including complete blood count, electrolyte panel, fasting lipid profile, and systemic C-reactive protein (CRP) were obtained per institutional protocol. Serum CRP concentrations were measured on a Hitachi 912 automated chemistry analyzer using a high-sensitivity polystyrene particle–enhanced immunoturbidimetric assay from DiaSorin (Stillwater, MN). Immediately following right femoral access, simultaneous left main coronary artery and coronary sinus blood samples were obtained before endothelial function assessment and stored at −80°C until assay. Left main and coronary sinus suPAR and PAI-1 levels were measured, and trans–left anterior descending coronary artery (LAD) biomarker gradients were calculated as described below. The suPAR concentrations were measured using Human suPAR ELISA Kit (MyBioSource, San Diego, CA; MBS2516189). PAI-1 concentrations were measured using MILLIPLEX Human Adipokine Magnetic Bead Panel 1 (EMD Millipore Corporation, Burlington, MA; HADK1MAG-61K).

Measurement of Local Coronary suPAR and PAI-1 Production

The gradients of suPAR and PAI-1 across the LAD circulation were calculated by subtracting the left main arterial concentration from the coronary sinus concentration. Net production of each biomarker in the LAD territory was then calculated, using a previously well-validated method, as the gradient times the coronary blood flow (CBF).17-21 For measurement of CBF in the LAD, a 6F or 7F guiding catheter was placed in the left main coronary artery, and a Doppler guidewire (Flowire, Volcano Therapeutics Inc, Rancho Cordova, CA) was positioned in the midportion of the LAD for blood velocity measurement.
measurements. Velocity signals were instantaneously obtained from the Doppler wire by an online fast Fourier transform, and average peak velocity was determined. Coronary artery diameter was measured by an independent investigator in the segment 5 mm distal to the tip of the Doppler wire offline with a quantitative coronary angiography program (Medis Corp, Leiden, the Netherlands) as previously described.\(^22\) CBF was calculated from the Doppler-derived time velocity integral and vessel diameter, where CBF=π×(coronary artery diameter/2)\(^2\)×(average peak velocity/2).\(^{23}\)

**Invasive Physiologic Assessment**

Endothelium-independent coronary flow reserve (CFR) in response to intracoronary adenosine administration and endothelium-dependent coronary vasoreactivity in response to intracoronary acetylcholine infusion were performed. In brief, intracoronary bolus injections of incremental doses (18-48 μg) of adenosine were administered until maximal hyperemia was achieved or the largest dose was given to evaluate CFR. CFR was calculated as the average hyperemic velocity postadenosine/average baseline velocity. Subsequently, to assess endothelium-dependent vasoreactivity, acetylcholine at increasing concentrations (10\(^{-6}\), 10\(^{-5}\), and 10\(^{-4}\) mol/L) was selectively infused into the LAD for 3 minutes at each concentration.\(^9,24\) Endothelium-independent microvascular dysfunction was defined as CFR <2.5 at maximal hyperemia after adenosine infusion.\(^9,25-27\) Coronary vasoreactivity was assessed as both epicardial endothelial function and endothelium-dependent microvascular function in response to acetylcholine. EED was defined as coronary artery vasoconstriction,\(^9,24,28,29\) with percentage change (Δ) in coronary artery diameter (D) <−20%, in response to intracoronary acetylcholine infusion.\(^30\) MED was defined as <50% ΔCBF in response to acetylcholine infusion.\(^{24,31-34}\)

**Statistical Analysis**

Continuous variables are described as mean±SD if normally distributed or median and interquartile range (IQR) if nonnormally distributed, and categorical variables as proportions. Student t test and chi-squared test were used for comparison of means or proportions for continuous and categorical variables, respectively. Nonnormally distributed variables were compared using the Mann-Whitney test and were log transformed as required. The associations between those variables were investigated using univariate and multivariate linear regression analyses after adjustment for traditional cardiovascular risk factors, including age, sex, race, diabetes mellitus, hypertension, dyslipidemia, and smoking. A 2-sided \(P<0.05\) was considered statistically significant. The statistical analyses were performed using SPSS 22 (IBM SPSS Statistics, Chicago, IL).

**Results**

Mean age was 53±10 years, 59 (75%) patients were women, 39 (49%) had hypertension, 49 (62%) had hyperlipidemia, 26 (33%) were current smokers, and 2 (3%) had diabetes mellitus. A majority, 71 (89.9%), of study patients were white, 2 (2.5%) were black, 1 (1.3%) was Hispanic, 1 (1.3%) was Native American, and 4 (5.1%) were other. Among all patients, 22 (28%) had endothelium-independent microvascular dysfunction with CFR <2.5 after adenosine, 49 (62%) patients had MED with ΔCBF <50% after acetylcholine, and 22 (26%) patients had EED with %ΔD <−20% after intracoronary acetylcholine infusion. Baseline demographic, laboratory, and clinical characteristics, as well as resting CBF, were not significantly different between patients with and those without MED (Table 1). Medication profiles were also similar between the 2 groups except for higher frequency of \(β\)-blocker usage in patients with MED (84% versus 16%, \(P=0.01\)). Baseline demographic, laboratory, and clinical characteristics were not different, except age (57±9 versus 51±10 years, \(P=0.02\)), which was higher in patients with than in those without EED (Table 1). Resting CBF was not significantly different between the 2 groups. Similarly, medication profiles were similar in both groups of patients with and without EED. Median local coronary suPAR production rate was 14.9 (IQR 86.9; −38.0) ng/min, and median PAI-1 production rate was 126.3 (IQR 5012.8; −2518.7) ng/min.

Patients with MED demonstrated a median local coronary suPAR production of 25.8 (IQR 121.6; −23.7) ng/min versus local suPAR extraction, observed in patients with normal microvascular function, of −12.7 (IQR 52.0; −74.8) ng/min; \(P=0.03\) (Figure 1). Moreover, as compared with all other patients, patients with isolated MED also had significantly higher local median coronary suPAR production (30.9 [122.2, −16.2] versus −9.6 [48.7, −74.8] ng/min; \(P=0.026\)). There was no significant difference between arterial systemic suPAR or coronary sinus suPAR levels between patients with versus those without MED (Table 2). Furthermore, there was no significant difference in median systemic CRP level (\(P=0.11\)) or local coronary PAI production (\(P=0.93\)) between patients with and those without MED. We did not observe a significant univariate linear relationship between local coronary suPAR production and %ΔCBF (\(r=0.12, P=0.28\)), nor was suPAR an independent predictor of %ΔCBF in a multivariate regression analysis after adjusting for age, sex, hypertension, diabetes mellitus, hyperlipidemia, body mass index, and smoking status.

Patients with EED had significantly higher median local coronary PAI-1 production as compared with local coronary PAI-1 extraction in those patients with normal epicardial endothelial function (1224.7 [IQR 12 940.7; −1915.4] versus −187.4 [IQR 4444.7; −4535.8] ng/min, \(P=0.03\)) (Figure 2).
There was no significant difference in arterial systemic PAI-1 or coronary sinus PAI-1 levels between patients with and those without EED (Table 2). Similarly, there was no significant difference in median systemic CRP level ($P=0.79$) or local coronary suPAR production ($P=0.80$) between patients with and those patients without EED. There was no significant linear relationship between PAI-1 production rate and $%\Delta D$. Age ($P=0.01$) and current smoking ($P=0.02$) were the only independent predictors of EED when age, sex, hypertension, diabetes mellitus, hyperlipidemia, body mass index, smoking, and PAI-1 production rate were accounted for.

Finally, median local coronary suPAR production ($P=0.80$), median local coronary PAI-1 production ($P=0.79$), and median systemic CRP level ($P=0.58$) were not significantly different between patients with (22 [28%]) and those patients without (57 [72%]) endothelium-independent microvascular dysfunction.

**Discussion**

This study shows that humans with early atherosclerosis and endothelial dysfunction have higher local cross-coronary suPAR and PAI-1 production. Increased local coronary suPAR production in the coronary circulation of patients with MED implicates immune and fibrinolytic pathways in the development of coronary endothelial dysfunction and increased cardiac events. Increased local coronary production of PAI-1 in patients with EED suggests that impaired or ineffective local fibrinolysis in response to possible subclinical coronary thrombosis occurs concomitantly with endothelial dysfunction in the development of CAD.
Local Coronary suPAR Production and Endothelial Dysfunction

The study shows, for the first time, that suPAR is locally released in the coronary circulation of patients with MED and extracted from the coronary arteries of those with normal microvascular function. The association between elevated suPAR levels and non–endothelium-dependent microvascular dysfunction has been previously reported. However, this is the first study to link local coronary suPAR production to endothelial dysfunction at the microvascular level and to suggest that cross-coronary suPAR production may be a useful biomarker of early atherosclerosis and MED.

suPAR has been extensively studied as a biomarker of inflammation and immune activation. Coronary microvascular dysfunction and elevated levels of systemic suPAR have both been associated with increased major adverse coronary outcomes. Elevated levels of suPAR have also been observed in advanced atherosclerotic disease in both the carotid and renal arteries, which in turn has been associated with major adverse coronary outcomes. This study links endothelial dysfunction, and therefore early atherosclerosis, with cross-coronary suPAR production and potentially with a higher risk of adverse cardiac events.

At the molecular level, Mustjoki et al demonstrated that both unstimulated blood mononuclear and endothelial cells can release suPAR. However, suPAR release is markedly enhanced when either mononuclear cells or thrombocytes were cultured with endothelial cells. Importantly, thrombocytes cultured in the absence of endothelium demonstrated no suPAR release, whereas coculture of the above-mentioned cell types without cell-cell contacts failed to enhance suPAR release, both suggestive of an important role for the endothelial vascular cell layer in suPAR release. Other molecular studies have shown that only 10% to 20% of activated urokinase plasminogen activator receptor is secreted as suPAR from the endothelial apical surface into the blood stream while the remaining activated urokinase plasminogen activator receptor on the basolateral side of the endothelial cells is secreted into the vessel wall where activated urokinase plasminogen activator/urokinase

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**Table 2. suPAR and PAI-1 Left Main and Coronary Sinus Concentrations as Well as Local Coronary Production Rates**

|                          | All Patients (n=79) | Normal Microvascular Endothelial Function 30 (38%) | Microvascular Endothelial Dysfunction 49 (62%) | Normal Epicardial Endothelial Function 58 (74%) | Epicardial Endothelial Dysfunction 20 (26%) |
|--------------------------|--------------------|--------------------------------------------------|-----------------------------------------------|------------------------------------------------|---------------------------------------------|
| **Left main suPAR**      |                    |                                                 |                                               |                                                 |                                             |
| concentration, ng/mL     | 9.7 (7.7, 11.1)    | 9.6 (7.1, 11.2)                                 | 9.7 (7.7, 11.3)                               | 9.7 (7.6, 11.5)                                 | 9.9 (7.9, 10.6)                            |
| **Coronary sinus suPAR** |                    |                                                 |                                               |                                                 |                                             |
| concentration, ng/mL     | 9.9 (8.0, 12.1)    | 9.0 (7.7, 11.0)                                 | 10.2 (8.2, 12.7)                              | 9.6 (7.9, 12.3)                                 | 10.0 (8.3, 11.2)                           |
| production rate, ng/min  | 14.9 (86.9, –38.0) | –12.7 (52.0, –74.8)                             | 25.8 (121.6, –23.7)                           | 12.8 (87.9, –50.2)                              | 11.6 (100.1, –36.3)                        |
| **Left main PAI-1**      |                    |                                                 |                                               |                                                 |                                             |
| concentration, ng/mL     | 166.1 (85.9, 281.5)| 125.0 (77.6, 214.2)                             | 198.0 (93.9, 300.3)                           | 166.1 (85.9, 275.3)                             | 156.3 (85.2, 302.2)                        |
| **Coronary sinus PAI-1** |                    |                                                 |                                               |                                                 |                                             |
| concentration, ng/mL     | 167.3 (84.0, 337.0)| 154.3 (52.0, 369.2)                             | 168.4 (100.8, 321.3)                          | 154.3 (89.0, 280.3)                             | 169.0 (103.7, 393.1)                       |
| production rate, ng/min  | 126.3 (5012.8, –2518.7) | 118.4 (4152.5, –1767.3) | 222.1 (6253.1, –3912.8) | –187.4 (4444.7, –4535.8) | 1224.7 (12 940.7, –1915.4) |

Numbers are medians (IQR). IQR indicates interquartile range; LAD, left anterior descending coronary artery; PAI-1, plasminogen activator receptor-1; suPAR, soluble urokinase plasminogen activator receptor.

*P<0.05 vs normal microvascular endothelial function.
†P<0.05 vs normal epicardial endothelial function.

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**Figure 2.** Cross-coronary PAI-1 production rate in patients with vs those without epicardial endothelial dysfunction. Bars represent medians and interquartile ranges. PAI-1 indicates plasminogen activator receptor-1.
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plasminogen activator receptor system is associated with macrophage foam cell formation, extracellular matrix degradation, and smooth muscle cell migration, all of which are associated with atherosclerotic plaque progression.43

Local coronary suPAR production in patients with MED and its extraction in those with normal microvascular function demonstrate that suPAR could possibly be a novel biomarker of early atherosclerosis and implicates inflammatory, immune, and fibrinolytic pathways in the development of coronary endothelial dysfunction and increased cardiac events.

The findings of this study are in line with our prior data linking local cross-coronary lipoprotein-associated phospholipase A2 production, a marker of inflammation, and increased oxidative stress secondary to altered anti-inflammatory function of the endothelium with early atherosclerosis in humans.19,20 Taken all together, these data provide another layer of evidence demonstrating the cardinal role of inflammation in the development of endothelial dysfunction and highlighting the potential important role of new anti-inflammatory therapy, such as canakinumab,44 in early coronary artery disease.

Local Coronary PAI-1 Production and Endothelial Dysfunction

This study shows that patients with EED and no obstructive coronary artery disease have higher local coronary PAI-1 production as compared with those with normal epicardial endothelial dysfunction. PAI-1, the main inhibitor of plasminogen activators, is a major inhibitor of fibrinolysis. It is produced and released from various cell types including adipocytes, platelets, macrophages, smooth muscle cells, and endothelial cells.45

Inhibition of fibrinolysis due to elevated PAI-1 levels has been associated with atherosclerotic plaque development, accelerated coronary atherosclerosis, thrombus formation, and an increased risk of recurrent myocardial infarctions.14,41,46-49 Moreover, elevated levels of PAI-1 have been documented in metabolic syndrome,50 diabetes mellitus,51 and obesity, all of which have been closely associated with endothelial dysfunction.

PAI-1 is not only a marker of ineffective fibrinolysis but is also a marker of abnormal vascular health. Adly et al52 showed that children and adolescents with diabetes mellitus type 1 who had microvascular complications including diabetic nephropathy, retinopathy, and neuropathy had significantly higher systemic PAI-1 levels. They also showed a significant positive linear relationship between PAI-1 level and carotid intima media thickness, a surrogate of early atherosclerosis. Results of our study are in line with those findings where higher local coronary PAI-1 production is associated with epicardial endothelial dysfunction and possibly increased subclinical coronary thrombosis.

Indeed, the differential association of studied biomarkers with MED (for suPAR) and EED (for PAI-1) may possibly be related to either different mechanisms underlying endothelial cell injury and/or different subsequent responses to endothelial cell injury at the epicardial and microvascular levels. Based on our data, 1 hypothesis would be that endothelial dysfunction at the microvascular level may be driven more by vascular inflammation and accumulation of inflammatory cells and/or fibrin deposits in the microcirculation, as reflected by suPAR production in patients with MED versus coronary suPAR extraction in those patients with normal microvascular function. A second hypothesis would be that significantly higher coronary PAI-1 production rate in patients with EED may not be directly related to the mechanism of endothelial cell injury at the epicardial level but rather be a reflection of increased subclinical thrombus formation associated with epicardial endothelial injury in the coronary circulation of “vulnerable patients,” those with a thrombotic vascular milieu. A third hypothesis would be that PAI-1 may contribute to thrombotic events in the epicardial vessels during acute coronary syndromes. Therefore, basic mechanistic studies should be undertaken to further elucidate the cause versus effect role of these novel biomarkers, given such a role’s significant implications for therapeutic and/or diagnostic value of these biomarkers in the future.

Study Limitations

Several limitations pertaining to this study should be noted. First, this is a small cross-sectional pilot study that only demonstrates an association between local coronary suPAR and PAI-1 biomarkers’ production and coronary endothelial dysfunction at the epicardial and microvascular levels. The detailed molecular mechanisms and possible causality among the local production of those biomarkers, immune and inflammatory pathways, early atherosclerosis, and coronary endothelial dysfunction all warrant further investigation. Second, a significantly higher number of patients with MED were on β-blockers as compared with those without MED. However, the fact that all patients were off vasoactive medications for 48 hours before the procedure negates any meaningful effect of this difference between the 2 groups. Finally, we do not have longitudinal follow-up data on our study patients to correlate cross-coronary biomarker production and endothelial dysfunction with hard cardiovascular outcomes. Prospective outcome studies are warranted to further evaluate this association.

Conclusion

The current study supports a potential role for suPAR and PAI-1 as biomarkers and potential mechanisms of endothelial dysfunction and reflects on the possible mechanisms...
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associated with its development in humans. This study shows for the first time that humans with early atherosclerosis have higher local cross-coronary suPAR and PAI-1 production. Increased local coronary suPAR production in the coronary circulation of patients with MED implicates immune and higher local cross-coronary suPAR and PAI-1 production. Increased local coronary production of PAI-1 in patients with EED suggests that impaired or ineffective local fibrinolysis occurs concomitantly with endothelial dysfunction in the development of CAD. Those findings could potentially have clinical implications, allowing for identification of patients with impaired coronary vasomotion and increased risk of CAD progression, who would benefit from initiation of early aggressive medical management. Furthermore, larger prospective clinical trials are warranted to investigate the magnitude of change in coronary suPAR and PAI-1 production rates, in conjunction with coronary endothelial function improvement, in response to lifestyle modifications such as weight loss, optimal traditional medical therapy, and novel pharmacological agents in patients with early CAD.

Disclosures

None.

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