Atherosclerosis is considered an inflammatory disease, initiated by the retention of low-density lipoprotein (LDL) in the arterial subendothelial space. LDL particles, trapped in the vessel wall, undergo modification and induce activation of endothelial cells and recruitment of inflammatory cells through chemokines and adhesion molecules.

Accumulation of inflammatory cells will, through the secretion of matrix metalloproteinases, lead to degradation of stabilizing components of plaque extracellular matrix, as collagen, contributing to a more vulnerable plaque.

The fibrinolytic system, through the activation of plasminogen to plasmin, has also been implicated in extracellular matrix degradation. From an inactive proenzyme, plasminogen can be converted to plasmin by 2 plasminogen activators (PA): tissue-type PA and urokinase-type PA (uPA), which binds to a cellular uPA receptor (uPAR). The conversion of plasminogen to plasmin will lead to degradation of fibrin and the activation of matrix metalloproteinases.

uPAR is found in different cells, such as monocytes, neutrophils, activated T-lymphocytes, macrophages, endothelial cells, smooth muscle cells (SMC), and tumor cells. When uPAR is cleaved by elastase, matrix metalloproteinases, chymotrypsin, u-PA, or cathepsin G, the soluble uPAR (suPAR) is released in different body fluids, such as urine, blood, and cerebrospinal fluid. Plasma suPAR levels increase during inflammatory conditions, such as bacterial and viral infections, sepsis, and cancer, reflecting the activation of the immune system.

Key Words: atherosclerosis • carotid stenosis • cytokines • inflammation
been associated with increased incidence of cardiovascular events.25,26 These findings, together with the knowledge that suPAR is a stable protein, make suPAR interesting as a future biomarker in atherosclerosis.27 Therefore, in this study suPAR levels in human atherosclerotic carotid plaques were measured to evaluate whether suPAR is associated with the unstable plaque, prone to rupture, focusing on extracellular matrix, histological markers, and inflammatory cytokines and chemokines.

Methods

Patients

One hundred sixty-two patients who underwent carotid endarterectomy were included in this study. The clinical characteristics of the patients are summarized in Table 1. Indications for surgery were plaques associated with symptoms (amaurosis fugax, transient ischemic attack, or stroke, n=92) and stenosis >70% or plaques not associated with symptoms and stenosis >80% (n=70). Blood samples were collected on the day before surgery. Informed consent was given by each patient. The study was approved by the local ethical review board.

Sample Preparation

After surgical removal, the carotid plaques were snap-frozen in liquid nitrogen. For the histological analysis 1-mm-thick fragments were cut from the most stenotic region of the plaque. The remaining parts of the plaques were homogenized as previously described.28

Histology

Sections (8 µm) were fixed with Histochoice (Amresco, Solon, OH), dipped in 60% isopropanol, and then in 0.4% Oil Red O in 60% isopropanol (for 20 min) to stain lipids. Masson trichrome using Ponceau-acid fuchsin (Chroma-Gesellschaft, Schimdt GmbH, Germany), Phosphomolybdic Acid, and Fast Green (Sigma Aldrich Chemie Gmbh, Germany) were used to assess plaque collagen content. When staining for macrophages, primary antibody monoclonal mouse anti-human CD68 (DakoCytomation, Glostrup, Denmark), diluted in 1% rabbit serum 1:100, and secondary antibody polyclonal rabbit anti-mouse (DakoCytomation, Glostrup, Denmark), diluted 1:200 in 1% of rabbit serum, were used. When staining for vascular smooth muscle cells (α-actin) primary antibody monoclonal mouse anti-human smooth muscle actin clone 1A4 (DakoCytomation, Glostrup, Denmark), diluted in 1% rabbit serum 1:50, and secondary antibody biotin rabbit anti-mouse Ig (DakoCytomation, Glostrup, Denmark), dilution 1:200 in 1% of rabbit serum, were used. For suPAR assessment primary antibody monoclonal mouse anti-human suPAR (DakoCytomation, Glostrup, Denmark), diluted in 1% rabbit serum 1:50, and secondary antibody polyclonal rabbit anti-mouse (DakoCytomation, Glostrup, Denmark), dilution 1:200 in 1% of rabbit serum, were used. The presence or absence of a ruptured fibrous cap was evaluated on the plaque sections stained for Oil Red O and Masson trichrome.

Areas of the different stainings in the plaque (% area) were quantified blindly using Biopix iQ 2.1.8 (Gothenburg, Sweden) after scanning with ScanScope Console Version 8.2 (LRI imaging AB, Vista Californien, USA) and photographed with Aperio image scope v.8.0 (Aperio, Vista Californien, USA).

Measurement of suPAR in Human Carotid Plaques and Plasma

Quantitative determination of suPAR in carotid plaques homogenates and plasma was done using suPARnostic Standard ELISA Kit (ViroGates, Denmark). For measurements in plaque homogenates, aliquots of plaque homogenate (50 µL) were centrifuged at 13 000g for 10 minutes. The supernatant (25 µL) was removed and used for measuring the amount of suPAR. suPAR standards (1.0–20.7 ng/mL), blank, curve control (2.6–3.4 ng/mL), and patient samples were mixed with peroxidase-conjugate monoclonal mouse anti-suPAR antibody and added to a clear microwell plate, pre-coated with a monoclonal antibody biotin rabbit anti-mouse Ig (DakoCytomation, Glostrup, Denmark), diluted in 10% of rabbit serum 1:100, and secondary antibody polyclonal rabbit anti-mouse (DakoCytomation, Glostrup, Denmark), diluted 1:200 in 1% of rabbit serum, were used. For suPAR assessment primary antibody monoclonal mouse anti-human suPAR (DakoCytomation, Glostrup, Denmark), diluted in 1% rabbit serum 1:50, and secondary antibody polyclonal rabbit anti-mouse (DakoCytomation, Glostrup, Denmark), dilution 1:200 in 1% of rabbit serum, were used. The presence or absence of a ruptured fibrous cap was evaluated on the plaque sections stained for Oil Red O and Masson trichrome.

Table 1. Clinical Characteristics of the Patients (n=162)

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic, n=70</th>
<th>Symptomatic, n=92</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>70.9 (SD 9.5)</td>
<td>66.2 (SD 6.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27 (SD 4.1)</td>
<td>26.8 (SD 4.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>48/22</td>
<td>59/33</td>
<td>NS</td>
</tr>
<tr>
<td>Degree of stenosis, %</td>
<td>90 (IQR 80–95)</td>
<td>90 (IQR 75–95)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>63</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>80</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (in the past or currently), %</td>
<td>83</td>
<td>76</td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>99</td>
<td>95</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting lipoproteins, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.3 (IQR 3.5–5.03)</td>
<td>4.3 (IQR 3.6–5.1)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.2 (IQR 1.8–3.1)</td>
<td>2.4 (IQR 2–3.4)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.1 (IQR 0.9–1.4)</td>
<td>1.1 (IQR 0.9–1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides cholesterol</td>
<td>1.3 (IQR 0.9–1.9)</td>
<td>1.3 (IQR 1–1.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>142 (SD 12.4)</td>
<td>138 (SD13.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinin, mmol/L</td>
<td>84 (IQR 71–97)</td>
<td>88 (IQR 76.3–101)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2 (IQR 0–4)</td>
<td>4 (IQR 2–8)</td>
<td>&lt;0.012</td>
</tr>
<tr>
<td>High-sensitive CRP</td>
<td>3.1 (IQR 2.9–6)</td>
<td>4.4 (IQR 2.2–7.4)</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cell count, 10^9/L</td>
<td>8.1 (SD 6.6–9.4)</td>
<td>7.7 (IQR 6.4–9.2)</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c, mmol/L</td>
<td>6.3 (IQR 5.5–7.03)</td>
<td>6.4 (IQR 5.5–7.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Statins, %</td>
<td>91</td>
<td>86</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as percentages, mean with SD, or when not normally distributed as median with interquartile range (IQR). Nonsignificant results are marked NS. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; CRP, c-reactive protein; HbA1c, hemoglobin A1c.
rat anti-human suPAR antibody. 3.3’,5.5’-tetramethylbenzidine (TMB) was added to the wells and incubated for 20 minutes in the dark. The absorbance at 450 nm, reference filter 650 nm, was measured with TECAN sunrise Absorbance Reader, Magellan, version 6.4 (Tecan Austria GmbH). The sample concentration was determined by interpolation on the standard curve and normalized to wet weight of plaques.

Cytokine Assessment
Aliquots of plaque homogenate (50 µL) were centrifuged at 13,000g for 10 minutes. The supernatant (25 µL) was removed and used for measuring interleukin (IL)-12 (p70), IL-12 (p40) IL-1β, IL-6, Fractalkine, interferon-γ (IFN-γ), IL-10, monocyte chemoattractant protein (MCP-1), monocyte inflammatory protein 1β (MIP-1β), platelet-derived growth factor AB/BB (PDGF-AB/BB), regulated on activation normal T-cell expressed and secreted (RANTES), vascular endothelial growth factor (VEGF), sCD40L, eotaxin, and tumour necrosis factor α (TNF-α). The procedure was performed according to the manufacturer’s instructions (Human Cytokine/chemokine immunoassay, Millipore Corporation, MA) and analyzed with Luminex 100 IS 2.3 (Austin, TX).

Quantitative Real-Time PCR on Human Plaque
Plaques were homogenized and total RNA extracted as described previously.29 Total RNA integrity and quantity were analyzed on BioRad Experion and Thermo Scientific NanoDrop instruments. Total RNA from each patient (500 ng) was reverse transcribed with SuperScriptIII RT and random primers (Invitrogen). Expression of ACTB (beta actin gene) (HS0106665_g1), PLAUR (uPAR gene) (HS00182181_m1), and CD3D (HS00174158_m1) was analyzed with FAM-labeled TaqMan Assays (Applied Biosystems) and a 7900HT real-time PCR System (Applied Biosystems). The relative quantity of mRNA expression of PLAUR and CD3D was calculated from standard curves, constructed by serial dilution of gel-purified PCR products, and using ACTB as the endogenous control. CD4 (HS00181217_m1) mRNA expression analysis was performed similarly, but using GAPDH (4326317E) and Cyclophilin A (4326316E) as endogenous controls. Ct values were determined in RQ Manager 1.2.1.

Statistics
Results were normalized to plaque wet weights, except CD3, CD4, and uPAR mRNA, which were normalized to the expression of housekeeping genes. Variables are presented as mean (standard deviation, SD), median (inter-quartile range, IQR), or percentages. Comparisons between groups were done using Mann–Whitney test, and Spearman rho was used to determine correlations. Significance was considered at P<0.05. SPSS 20.0 was used for the statistical analysis.

Results
suPAR, uPAR, and Clinical Events
The suPAR content (µg/g wet weight plaque) was significantly higher in plaques associated with symptoms compared with asymptomatic plaques (0.041 [IQR 0.016–0.059] vs. 0.022 [IQR 0.012–0.038], P=0.001; Figure 1A). Circulating suPAR levels (ng/mL) were higher in plasma from symptomatic patients compared with plasma from asymptomatic patients (5.04 [IQR 4.18–6.54] vs. 4.61 [IQR 3.63–5.70], P=0.026; Figure 1B). No significant association was found between plasma and plaque levels of suPAR in the same patient.

No significant differences were found comparing uPAR mRNA expression or histologically stained area of uPAR between symptomatic and asymptomatic plaques.

suPAR, uPAR, and the Vulnerable Plaque
Plaque levels of suPAR correlated with the area stained for lipids (ORO, % of total plaque area; Figure 2A), as well as with the area of macrophages (CD68 staining, % of total plaque area; Figure 2B). There was also a negative correlation between plaque levels of suPAR and α-actin (Figure 2C), the marker used to assess SMC. No correlations were found between plaque levels of suPAR and collagen. suPAR measured in the plasma also correlated with uPAR mRNA (r=0.286, P=0.001) and uPAR measured histologically (r=0.371, P<0.001). No associations were found between plasma levels of suPAR and histology.

uPAR in plaque measured histologically (% of area) correlated with uPAR mRNA in the plaque (r=0.342, P<0.001), uPAR staining (Figure 3A) as well as mRNA correlated with staining for macrophages (Table 2, Figure 3D), lipids (Table 2, Figure 3H), and inversely to smooth muscle cell staining (Table 2, Figure 3I). An inverse relationship was also found between uPAR measured histologically and Masson trichrome staining for collagen (Table 2, Figure 3G).

Figure 1. A. Mean levels of soluble urokinase plasminogen activator receptor (suPAR) (µg/g) in symptomatic plaques compared with asymptomatic plaques. B. Mean levels of suPAR (ng/mL) in plasma from symptomatic patients compared with asymptomatic patients.
No significant differences were found between plaques with a ruptured or an intact fibrous cap in what concerned plaque levels of suPAR or plaque levels of uPAR. Although no differences were found in plaque levels of suPAR or uPAR, plasma levels of suPAR were significantly increased in patients with a ruptured fibrous cap when compared with those having plaques with intact fibrous caps (5.02 [IQR 4.10–5.92] vs. 4.29 [IQR 3.64–5.24], \( P = 0.018 \)).

Both uPAR mRNA levels and uPAR assessed histologically correlated with mRNA levels of CD4 (Table 2) and CD3 (Table 2) in the plaque. SuPAR levels in plaque correlated with CD4 mRNA in the plaque (Table 2). Plasma levels of suPAR correlated inversely with CD3 mRNA measured in the plaque (\( r = -0.218, P = 0.017 \)).

**suPAR, uPAR, and Plaque Inflammation**

Plaque suPAR correlated with proinflammatory chemokines and cytokines in the plaque including MCP-1, TNF-\( \alpha \), IL-1\( \beta \), IL-6, PDGF-AB/BB, MIP-1\( \beta \), RANTES, and s-CD40L (Table 3). Furthermore, suPAR correlated with plaque levels of the anti-inflammatory cytokine IL-10 (Table 3). Finally, an inverse correlation between the plaque content of eotaxin and suPAR was found (Table 3). No other significant correlations were found between suPAR and other cytokines measured. No significant correlations were found between plasma levels of suPAR and the inflammatory cytokines measured in the plaque.

uPAR measured histologically in the plaque correlated with IL-10, IL-1\( \beta \), IL-6, MCP-1, MIP-1\( \beta \), and RANTES measured in the plaque (Table 3). uPAR mRNA levels measured in the plaque correlated with IL-6, IL-1\( \beta \), and MIP-1\( \beta \) (Table 3). No other significant correlations were found.
suPAR, uPAR, and Clinical Risk Factors

Both plasma and plaque levels of suPAR correlated with age ($r=0.284$, $P<0.001$ and $r=0.165$, $P=0.036$, respectively). Plasma levels of suPAR in diabetics were significantly higher than in nondiabetics ($5.39$ [IQR 4.15–6.8] vs $4.63$ [IQR 3.86–5.72], $P=0.015$). Plasma levels of suPAR were lower in plasma from males compared with females ($5.47$ [IQR 4.61–6.65] vs $4.5$ [IQR 3.83–5.7], $P=0.002$).

Furthermore plaque levels of suPAR correlated with plasma levels of LDL ($r=0.190$, $P=0.021$) and inversely with plasma levels of high-density lipoprotein ($r=-0.193$, $P=0.018$). uPAR measured histologically correlated with circulating LDL levels ($r=0.197$, $P=0.017$).

Plasma levels of suPAR showed significant correlations with C-reactive protein (CRP), high-sensitive CRP, and creatinine ($r=0.268$, $P=0.001$, $r=0.216$, $P=0.008$, and $r=0.236$, $P=0.004$, respectively).

No significant differences when comparing patients with or without statin treatment were found in plasma or plaque levels of suPAR or uPAR levels.

Discussion

Lately suPAR has gained interest as a possible marker for vulnerable plaques, even though the association between plaque inflammation and suPAR levels remains unknown. This is the first study to show that plaque levels of suPAR correlate with increased inflammatory activity in the plaque as well as with the vulnerable phenotype of the human carotid plaque.

SuPAR, uPAR, and the Vulnerable Plaque

Vulnerable plaques have a core of lipids, a thin fibrous cap, infiltration of inflammatory cells in the fibrous cap, remodeling, and increased neovascularization. LDL, retained in the vessel wall, are modified through different mechanisms such as oxidation. Oxidized LDL trapped in the vessel wall activates the endothelium and leads to recruitment of inflammatory cells, such as monocytes and macrophages, contributing to the inflammatory process and enhanced atherosclerotic process. uPAR, known to be present on such inflammatory cells as monocytes and macrophages, does also contribute to the inflammatory process through different actions as chemotaxis, cell migration, and cell adhesion. When exposed to proteases, metalloproteinases, and elastases released from activated monocytes and macrophages, cell-surface–bound uPAR is cleaved, resulting in the release of suPAR.

This study shows that plaque levels of uPAR and suPAR correlate with levels of macrophages and lipids in the plaque. These positive correlations might be explained in 2 different ways. A large amount of lipids in the vessel wall

Table 2. Correlations Between Plaque Components and Plaque Levels of uPAR Assessed With PCR (mRNA) and Histologically, as Well as suPAR (µg/g Wet Weight Plaque), Measured by ELISA

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Plaque-suPAR (mRNA)</th>
<th>Plaque uPAR (% area)</th>
<th>Plaque suPAR (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>$r=0.274^{***}$</td>
<td>$r=0.439^{***}$</td>
<td>$r=0.507^{***}$</td>
</tr>
<tr>
<td>CD68</td>
<td>$r=0.311^{***}$</td>
<td>$r=0.467^{***}$</td>
<td>$r=0.294^{***}$</td>
</tr>
<tr>
<td>Alpha-actin</td>
<td>$r=-0.205^*$</td>
<td>$r=-0.322^{***}$</td>
<td>$r=-0.253^{***}$</td>
</tr>
<tr>
<td>Masson trichrome</td>
<td>NS</td>
<td>$r=-0.176^*$</td>
<td>NS</td>
</tr>
<tr>
<td>CD3 (mRNA)</td>
<td>$r=0.887^{***}$</td>
<td>$r=0.191^*$</td>
<td>NS</td>
</tr>
<tr>
<td>CD4 (mRNA)</td>
<td>$r=0.337^{***}$</td>
<td>$r=0.409^*$</td>
<td>$r=0.308^{***}$</td>
</tr>
</tbody>
</table>

suPAR indicates soluble urokinase plasminogen activator receptor; uPAR, urokinase plasminogen activator receptor; NS, nonsignificant.

Table 3. Correlations Between Plaque and Plasma Levels of suPAR, uPAR (mRNA Expression and % of Plaque Area), and Cytokines/Chemokines (µg/g) Measured in the Plaque

<table>
<thead>
<tr>
<th>Cytokine/Chemokine (µg/g)</th>
<th>Plaque-suPAR (µg/g)</th>
<th>Plasma-suPAR (ng/mL)</th>
<th>uPAR mRNA</th>
<th>uPAR (% area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>$r=0.377^{***}$</td>
<td>NS</td>
<td>$r=0.248^{**}$</td>
<td>$r=0.214^{**}$</td>
</tr>
<tr>
<td>IL-6</td>
<td>$r=0.575^{***}$</td>
<td>NS</td>
<td>$r=0.182^*$</td>
<td>$r=0.233^{***}$</td>
</tr>
<tr>
<td>MCP-1</td>
<td>$r=0.608^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>$r=0.220^{**}$</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>$r=0.565^{***}$</td>
<td>NS</td>
<td>$r=0.323^{***}$</td>
<td>$r=0.227^{**}$</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$r=0.236^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-10</td>
<td>$r=0.334^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>$r=0.199^{*}$</td>
</tr>
<tr>
<td>RANTES</td>
<td>$r=0.404^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>$r=0.179^*$</td>
</tr>
<tr>
<td>PDGF-AB/BB</td>
<td>$r=0.256^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>$r=0.377^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>s-CD40L</td>
<td>$r=0.194^*$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

suPAR indicates soluble urokinase plasminogen activator receptor; uPAR, urokinase plasminogen activator receptor; IL-1β, interleukin-1β; NS, nonsignificant; MCP-1, monocyte chemoattractant protein; MIP-1β, monocyte inflammatory protein 1β; TNF-α, tumour necrosis factor α; RANTES, regulated on activation normal T-cell expressed and secreted; PDGF-AB/BB, platelet-derived growth factor AB/BB.

Significance marked by *$P<0.05$, **$P<0.01$, and ***$P<0.005$. 

suPAR, uPAR, and the Vulnerable Plaque

Vulnerable plaques have a core of lipids, a thin fibrous cap, infiltration of inflammatory cells in the fibrous cap, remodeling, and increased neovascularization. LDL, retained in the vessel wall, are modified through different mechanisms such as oxidation. Oxidized LDL trapped in the vessel wall activates the endothelium and leads to recruitment of inflammatory cells, such as monocytes and macrophages, contributing to the inflammatory process and enhanced atherosclerotic process. uPAR, known to be present on such inflammatory cells as monocytes and macrophages, does also contribute to the inflammatory process through different actions as chemotaxis, cell migration, and cell adhesion. When exposed to proteases, metalloproteinases, and elastases released from activated monocytes and macrophages, cell-surface–bound uPAR is cleaved, resulting in the release of suPAR.

This study shows that plaque levels of uPAR and suPAR correlate with levels of macrophages and lipids in the plaque. These positive correlations might be explained in 2 different ways. A large amount of lipids in the vessel wall
will, as described above, attract such inflammatory cells as macrophages and activated lymphocytes, which both express uPAR. Agglomerates of activated inflammatory cells release proteolytic enzymes and inflammatory cytokines, generating a hostile environment leading to cell apoptosis and necrosis of stabilizing cells.31,32 uPAR on SMC and monocytes exposed to proteases will in turn lead to the cleavage of uPAR and thereby the release of suPAR as described above. This hypothesis is supported by the associations between suPAR, uPAR, CD3 mRNA, CD4 mRNA, and macrophages found in this study. Cleavage mechanism experiments were not performed in our study. It is known that metalloproteinases and other proteases that cleave suPAR are present in plaques.2,5,14–19,33,34 The inflammatory activity in the plaque will attract even more monocytes/macrophages and T-cells. A possible explanation is that uPAR expressed on these cells could be cleaved through the action of different enzymes, which will result in the release of suPAR. In this study no significant associations were found between plaque and plasma levels of suPAR, even though both plasma and plaque levels of suPAR were increased in symptomatic patients. A study by Chavakis et al34 showed that the release of suPAR from human umbilical vein endothelial cells mostly appeared at the basolateral side of the membrane rather than to the apical side. This could correspond in human atherosclerosis to a release into the plaque-media-adventitia rather than to the lumen-circulation, resulting in increased suPAR levels in the plaque not reflected in plasma. On the other hand, when there is a rupture in the fibrous cap, according to our results there is an association with increased plasma levels of suPAR. At this point blood penetrates the tissue, and therefore plasma suPAR will be able to be increased. The associations between suPAR, uPAR, and inflammatory cells might also be explained directly as responses to inflammatory cytokines released from activated monocytes and lymphocytes. Cytokines stimulate the release of suPAR from neutrophils, endothelial cells, and SMCs or directly through the cleavage of membrane-bound uPAR on monocytes.35,36 Additionally, higher amounts of suPAR have been suggested as a contributor to the inflammatory process through its potential role as a chemotactic agent.16,36–38 Our study showed significant correlations between both suPAR and uPAR measured in the plaque with macrophages and CD4 mRNA and between uPAR and CD3 mRNA, suggesting these inflammatory cells as potential sources of uPAR for cleavage to suPAR, as a result of the ongoing inflammatory process in the plaque. This study does not prove any causal relationship between suPAR, uPAR levels, and the cleavage of uPAR, even though the current findings are supported by earlier in vitro studies focusing on uPAR cleavage. The present study showed a negative correlation between SMC and suPAR. In a study by Svensson et al25 uPAR was shown to correlate with macrophages and that its distribution in the plaque was uneven with high concentrations near the rupture site. In this study suPAR was also shown to correlate with macrophages in the plaque. The region of rupture is known to be rich in inflammatory cells, whereas SMCs are more absent as a result of inflammation causing apoptosis and necrosis of SMC.30–32 This means that suPAR levels increase with macrophages in the inflammatory plaque while SMC decreases, resulting in a thin protective cap and in plaques that are more prone to rupture (Figure 3). Our study did not show any differences in suPAR levels in the plaques with ruptured caps compared with plaques with intact caps. Because only a fragment of the plaque was used for histology, it is not certain that the exact rupture site was included in that fragment. This could be a limitation. However, to obtain homogenate and RNA of the rest of the plaque it was technically impossible to perform histology on the same material. These technical difficulties can also explain the lack of significance, but the presence of a tendency, when measuring uPAR mRNA in symptomatic versus asymptomatics, although a clearly significant difference was found for suPAR in these two groups.

Another hypothesis that would explain the inverse association between suPAR, uPAR, and SMC is that macrophages, endothelial cells, and T-cells produce cytokines and chemokines under inflammatory conditions that cause proliferation and migration of SMC from media to intima.39 Intima SMCs are different from media SMCs because those in the intima express lower levels of SMC α-actin (SMC-αA) and SMC heavy chain myosin (SMC-HCM). The finding in this study that SMC-αA area correlates negatively with suPAR levels might therefore be explained by the proliferation and migration of intima SMC with low levels of SMC-αA.

suPAR and Inflammation

Both uPAR and suPAR are, as mentioned above, associated with inflammation and many of the systemic inflammatory diseases such as cancer, rheumatoid arthritis, and atherosclerosis. Atherosclerosis is also known to progress under conditions with enhanced inflammatory activity.3,40,41 The fact that suPAR is not only associated with atherosclerosis but also with systemic inflammatory diseases has been considered a weakness concerning the use of suPAR as biomarker for atherosclerosis.

To evaluate whether suPAR and uPAR correlated with the local inflammatory process in the plaque, inflammatory cytokines were measured in plaques. suPAR has been shown, in a previous study by Pawlak et al,42 to correlate with MCP-1, MIP-1β, and RANTES in plasma. It has also before been shown that inflammatory cytokines as IL-1β and PDGF can induce the release of suPAR from endothelial cells and SMC and that TNF-α stimulates suPAR release from monocytes and IL-8 stimulates suPAR release from neutrophiles.35,36,43 In this study suPAR correlated with several of the main proinflammatory cytokines and chemokines, in plaque tissue, involved in the atherosclerotic process, including IL-6, MCP-1, MIP-1β, IL-1β, PDGF, RANTES, sCD-40L, and TNF-α. The same associations were shown for uPAR except for sCD-40L, PDGF, and TNF-α. Further, correlations were found between suPAR, uPAR, and the anti-inflammatory cytokine IL-10. IL-10 is present in the atherosclerotic lesion and is upregulated by oxidized LDL, even though it is considered an anti-inflammatory cytokine.44,45 This means that the correlation between suPAR and increasing levels of IL-10 might be explained as a counter-balancing factor, like in a feedback loop, to the ongoing inflammatory process.
These findings show that there is an association between suPAR and uPAR levels and the inflammatory activity in the atherosclerotic lesion. The results strengthen previous in vitro findings. However, whether suPAR is a contributor to or a result of the inflammatory process remains unknown.

**suPAR and Clinical Relevance**

Plasma levels of suPAR have been associated with increased risk of developing cardiovascular events. In this study no significant associations were found between plasma and plaque levels of suPAR, even though they were both increased in symptomatic patients. Plaque levels of suPAR correlated with age. This relation with age has been shown previously in plasma but not in plaques. In this study plasma levels of suPAR correlated with age, CRP, and creatinin and were increased in both symptomatics and diabetics (comparing with asymptomatics). Further studies are needed on the role of suPAR and risk for cardiovascular events.

**Conclusions**

This study is the first to show that suPAR in human carotid plaques and plasma is associated with the presence of symptoms. Additionally, the present study shows that the levels of suPAR in plaques are also associated with the vulnerable plaque phenotype and the inflammatory process in the plaque.

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**Disclosures**

None.

**References**


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