Reduction of Circulating Soluble Fms-Like Tyrosine Kinase-1 Plays a Significant Role in Renal Dysfunction–Associated Aggravation of Atherosclerosis

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Background—Renal dysfunction is commonly accompanied by a worsening of atherosclerosis; however, the underlying molecular mechanism is not fully understood. We examined the role played by soluble fms-like tyrosine kinase-1 (sFlt-1), an endogenous antagonist of the proatherogenic cytokine placental growth factor (PlGF), in the worsening of atherosclerosis in patients with renal dysfunction and in an animal model of renal failure.

Methods and Results—In this study, 329 patients who received cardiac catheterization and 76 patients who underwent renal biopsy were enrolled. Both plasma sFlt-1 levels and renal sFlt-1 mRNA expression were positively correlated with estimated glomerular filtration rate (P<0.01). The PlGF/sFlt-1 ratio was negatively correlated with estimated glomerular filtration rate (P<0.01), whereas plasma PI GF levels were not affected by it. The PlGF/sFlt-1 ratio was significantly higher in patients with multivessel coronary artery disease than in patients with single-vessel or no coronary artery disease. The reduction of circulating sFlt-1 and renal sFlt-1 mRNA levels was confirmed in five-sixths (5/6)–nephrectomized apolipoprotein E–deficient mice that developed experimental renal dysfunction. Atherosclerotic plaque area and macrophage infiltration into the plaque were significantly higher in 5/6–nephrectomized apolipoprotein E–deficient mice than in control mice, but replacement therapy with recombinant sFlt-1 significantly reduced both plaque formation and macrophage infiltration.

Conclusions—The present study demonstrates that a reduction in the circulating levels of sFlt-1 is associated with the worsening of atherosclerosis that accompanies renal dysfunction. (Circulation. 2009;120:2470-2477.)

Key Words: atherosclerosis • coronary disease • growth substances • heart failure • kidney

Chronic kidney disease is a worldwide public health problem not only because it leads to end-stage renal failure1–3 but also because it is an independent risk factor for atherosclerosis-related cardiovascular events.4,5 Accumulating evidence indicates that atherosclerosis is often worsened in patients with renal dysfunction,6–9 and the risk of cardiovascular disease increases sharply as the estimated glomerular filtration rate (eGFR) declines.10 Additionally, more than 50% of deaths among end-stage renal failure patients are due to cardiovascular events.11 Although it is clear that most cardiovascular events associated with renal dysfunction result from atherosclerosis, the underlying molecular mechanism responsible for the worsening of atherosclerosis in chronic kidney disease is not yet fully understood. Consequently, an effective therapeutic strategy is still lacking.

Clinical Perspective on p 2477
Fms-like tyrosine kinase 1 (Flt-1), which is a receptor tyrosine kinase and a member of the vascular endothelial growth factor (VEGF) receptor family,12 is a specific receptor for placental growth factor (PIGF) and VEGF-A. Soluble Flt-1 (sFlt-1), which consists of the 6 extracellular immunoglobulin-like domains of Flt-1, circulates as an endogenous antagonist of both PIGF and VEGF-A. PIGF is thought to exacerbate atherosclerosis by enhancing angiogenesis and the migration of monocytes/macrophages into the arterial wall.13 Consistent with that idea, administration of an antibody against Flt-1 exerts an antitherogenic effect in atherosclerosis-prone apolipoprotein E–deficient (apoE-deficient) mice by inhibiting the early growth of atheroscle-
Clinical Study

Patient Population

In the present study, 329 consecutive patients admitted to Nara Medical University Hospital to undergo diagnostic cardiac catheterization for angina pectoris or congestive heart failure or to undergo follow-up coronary angiography after myocardial infarction were enrolled for evaluation of the relationship among renal function, plasma sFlt-1 levels, and the severity of coronary artery disease. In addition, 76 patients admitted to the hospital to undergo renal biopsy to diagnose the cause of proteinuria or renal dysfunction were enrolled for investigation of renal expression of sFlt-1. The clinical parameters assessed included age, sex, coronary risk factors, body weight, and serum creatinine. We calculated eGFR using a modified Modification of Diet in Renal Disease equation: eGFR (mL · min⁻¹ · 1.73 m⁻²) = 194 × (serum creatinine)^−1.094 × age^-0.283 × 0.793 (if female). Clinical study protocols were approved by our institutional ethics committee (No. 2002-009, Nara Medical University Ethics Committee), and written informed consent was obtained in all cases from either the patient or his/her family members.

Cardiac Catheterization and Blood Sampling

Patients were recruited from February 2005 to March 2007. Those with acute coronary syndrome, evidence of malignant disease, or an unwillingness to participate were excluded. On the basis of the findings of coronary angiography and left ventriculography, a diagnosis was made, and the severity of coronary atherosclerosis was evaluated in terms of the number of vessels with >75% stenosis or Gensini’s scoring method15 (see the Methods section of the online-only Data Supplement for details). The severity of coronary artery disease was assessed by 2 independent angiographers who were blinded to the patients’ backgrounds. Patients with dilated cardiomyopathy, hypertrophic cardiomyopathy, hypertensive heart disease, and valvular heart disease were categorized as having congestive heart failure. Patients with vasospastic angina pectoris or chest pain syndrome were categorized as “other.”

At the beginning of the cardiac catheterizations, we collected blood samples before the injection of heparin. Samples were collected from the aortas of all enrolled patients, and samples from the coronary sinus, the hepatic vein, and the renal vein were collected simultaneously in 14 patients. Subsequently, plasma or serum samples were respectively added to EDTA anticoagulant tubes or plain tubes with serum separating agent and stored at −80°C until assayed. We measured plasma levels of sFlt-1 and PlGF and serum levels of VEGF using commercially available ELISA kits (DVR100B, DPG90, and DVE600, respectively; R&D Systems, Minneapolis, Minn; see the Methods section of the online-only Data Supplement for details).

Renal Biopsy and mRNA Analysis

Seventy-six consecutive patients who underwent diagnostic renal biopsy between May 2002 and October 2007 were enrolled. Patients with evidence of malignant disease or who were unwilling to participate were excluded. Renal specimens were stored at −80°C until assayed. We extracted mRNA from the specimens and generated cDNA as described previously.12 Relative sFlt-1 mRNA levels were then determined by quantitative real-time polymerase chain reaction with cDNA samples with primers 5′-CCCTGAAGAATCTCAGGACC-3′ (forward) and 5′-GAGCCTCTCCAGGACGCTG-3′ (reverse), which correspond to a unique sequence in the human sFlt-1 mRNA. Levels of sFlt-1 mRNA were normalized to those of GAPDH mRNA (see the Methods section of the online-only Data Supplement for details).

Experimental Study

We performed an experimental study to confirm the relationship between renal dysfunction and cardiovascular disease observed in the clinical study.

Animals

Male apoE-deficient mice (C57BL/6 background) were purchased from Taconic Farms (Hudson, NY) and maintained in a temperature-controlled room with a 12-hour light/dark cycle and free access to water and standard chow until they were 11 weeks old. Thereafter, they were maintained on a Western diet (16.5% fat, 1.25% cholesterol, 5% sodium cholate) until they were 22 weeks old. All experiments were approved by the Ethics Review Committee for Animal Experimentation of Nara Medical University.

Experimental Renal Failure and Injection of Recombinant Human sFlt-1 Replacement Therapy

At 8 weeks of age, mice were randomly assigned to a 5/6 nephrectomy (chronic renal failure; removal of one kidney and two thirds of the other kidney) or a control group. The 5/6 nephrectomy operation was performed as described previously.19–20 Recombinant human sFlt-1 (rhFlt-1, amino acids 1 to 338) was made from human Flt1 [sFlt-1 (D1–3)], which contains 3 immunoglobulin-like domains in its N-terminal region. The accuracy of production was confirmed in vitro and in vivo before the replacement therapy. Details can be found in the online-only Data Supplement. We administered rhFlt-1 intraperitoneally at a dose of 15 ng per gram of body weight 3 times per week for 10 weeks beginning when the mice were 12 weeks old. Control mice received phosphate-buffered saline (PBS) at the same intervals over the same period. The mice were euthanized at 22 weeks under general anesthesia with pentobarbital sodium, after which blood samples were collected from the right ventricle, and the serum was stored at −80°C until analyzed. The hearts, aortas, and kidneys were excised and stored likewise.

Blood and mRNA Analysis

Serum creatinine, total cholesterol, and triglyceride levels were assayed with enzymatic kits (Wako Pure Chemical Industries, Osaka, Japan). We also measured mouse sFlt-1, mouse PIGF-2, mouse VEGF, and human sFlt-1 using ELISAs (MVR100, MP200, MMV00, and DVR100B, respectively; R&D Systems). We extracted mRNA from frozen renal specimens and synthesized cDNA using standard protocols. Relative levels of mouse sFlt-1 mRNA were then determined by real-time polymerase chain reaction with cDNA samples with primers 5′-CTCTGAAGAATCTCAGGACC-3′ (forward) and 5′-GAGCCTCTCCAGGACGCTG-3′ (reverse), which corresponds to a unique sequence of mouse sFlt-1 mRNA. Levels of sFlt-1 mRNA were normalized to those of GAPDH mRNA.

Measurement of Atherosclerotic Lesions and Histological Examination

To quantify atherosclerotic plaque formation, atherosclerotic lesions within the thoracocaudal abdominal aorta were stained with oil red O, and thin slices of aortic root (Valsaiva sinus) were stained with Masson’s trichrome. The aortic root slices were also labeled with anti-monocyte/macrophage antibody (MOMA-2; ABR Affinity Bioreagents, Golden, Colo). Plaque areas and MOMA-2–stained areas were traced by 2 independent examiners who were blinded to the specimens’ background and were measured with ImageJ version 1.41 software (http://rsb.info.nih.gov/ij/) as described previously.21
Table 1. Clinical Characteristics of the Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cardiac Catheterization and Blood Analysis</th>
<th>Renal Biopsy and mRNA Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eGFR ≥60 mL·min⁻¹·1.73 m⁻²</td>
<td>eGFR &lt;60 mL·min⁻¹·1.73 m⁻²</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>153 (74) (n=207)</td>
<td>86 (71) (n=122)</td>
</tr>
<tr>
<td>Age, y (mean±SD)</td>
<td>64±10</td>
<td>69±10‡</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>112 (54)</td>
<td>96 (79‡)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>117 (57)</td>
<td>51 (42†)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>74 (36)</td>
<td>45 (37)</td>
</tr>
<tr>
<td>Smoking</td>
<td>130 (63)</td>
<td>76 (62)</td>
</tr>
<tr>
<td>Obesity</td>
<td>82 (40)</td>
<td>33 (27)*</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>29 (14)</td>
<td>17 (14)</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>44 (21)</td>
<td>40 (33)*</td>
</tr>
<tr>
<td>Old myocardial infarction</td>
<td>88 (43)</td>
<td>45 (37)</td>
</tr>
<tr>
<td>Other</td>
<td>46 (22)</td>
<td>20 (16)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>IgA glomerular nephritis</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Minimal-change nephrotic syndrome</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Minor change</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01, ‡P<0.001 vs eGFR ≥60 mL·min⁻¹·1.73 m⁻².

Statistical Analysis

Continuous data are expressed as mean±SEM unless otherwise indicated. The significance of differences between 2 groups was determined with the Student t test and that between more than 3 groups was determined with 1-way ANOVA. Post hoc pairwise comparisons were performed with the Tukey-Kramer test in the clinical study and the Bonferroni/Dunn test in the experimental study. To assess correlations between 2 continuous variables, Pearson’s correlation coefficient analysis and simple linear regression were performed. Multiple linear regression was performed to determine the variables that affected the sFlt-1 value. Values of P<0.05 were considered statistically significant. All statistics were calculated with Stat View for Windows, version 5.0 (SAS Institute Inc., Cary, NC).

Clinical Study

Patient Characteristics

The clinical characteristics of the patients receiving cardiac catheterization and blood analysis are shown in Table 1. Among the patients, 27 were receiving maintenance hemodialysis. The characteristics of the patients enrolled in the renal biopsy study are also shown in Table 1. This study included no patients receiving hemodialysis.

Plasma sFlt-1 Levels

We analyzed sFlt-1 levels in plasma obtained from the aorta. A significant positive correlation was found between sFlt-1 levels in plasma from the aorta and eGFR (r=0.32, P<0.001; Figure 1A). Multiple linear regression revealed the sFlt-1 levels were not influenced by age, sex, or coronary risk factors but were affected only by eGFR (Table 2). To determine the site of sFlt-1 production, we measured sFlt-1 levels in plasma collected from the aorta, coronary sinus, hepatic vein, and renal vein of 14 patients. Among these 4 vessels, sFlt-1 levels were significantly higher in the renal vein than in any of the others (aorta, coronary sinus, hepatic vein, and renal vein: 325.6±43.4, 336.6±39.8, 172.3±67.8, 247.5±43.4 mL·min⁻¹·1.73 m⁻², respectively).

Figure 1. Plasma sFlt-1 and PlGF levels and expression of sFlt-1 mRNA. A, A significantly positive correlation was found between plasma levels of sFlt-1 from aorta and eGFR, n=329; r=0.32; P<0.001. B, A significantly positive correlation was found between relative levels of sFlt-1 mRNA in human renal biopsy specimens and eGFR, n=76; r=0.25; P=0.032. C and D, Relationship between plasma sFlt-1 and PlGF levels and coronary atherosclerosis. PlGF/sFlt-1 ratios plotted against eGFR (C) and extent of coronary atherosclerosis (D). A significantly negative correlation was found between PlGF/sFlt-1 ratio and eGFR (r=−0.27; P<0.001). Furthermore, PlGF/sFlt-1 ratio was significantly different according to the number of coronary arteries that showed >75% stenosis. Ninety-five percent confidence interval is also presented in A–C. *P<0.05 vs patients without coronary artery stenosis. Data are mean±SEM in D.
Affecting the Value of sFlt-1

Table 2. Multiple Linear Regression to Assess Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>95% Confidence Interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>2.85</td>
<td>1.96–3.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>1.51</td>
<td>−0.82 to 3.84</td>
<td>0.204</td>
</tr>
<tr>
<td>Male sex</td>
<td>−23.2</td>
<td>−84.3 to 38.0</td>
<td>0.456</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11.0</td>
<td>−37.2 to 59.1</td>
<td>0.655</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>4.39</td>
<td>−41.0 to 49.8</td>
<td>0.849</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.12</td>
<td>−38.0 to 54.2</td>
<td>0.729</td>
</tr>
<tr>
<td>Smoking</td>
<td>−13.3</td>
<td>−68.4 to 41.9</td>
<td>0.636</td>
</tr>
<tr>
<td>Obesity</td>
<td>8.86</td>
<td>−38.5 to 56.2</td>
<td>0.713</td>
</tr>
</tbody>
</table>

If a patient was male or had the disease or the habit, then 1 was given as the variable; if not, then 0 was given as the variable.

and 692.5±115.8 pg/mL, respectively; P<0.001; online-only Data Supplement Figure IA), which indicates that the kidney is a possible source of circulating sFlt-1. Moreover, there was a strong correlation between sFlt-1 levels in plasma from the aorta and plasma from the renal vein (n=126, r=0.70, P<0.001; online-only Data Supplement Figure IB).

Specific Expression Profile of sFlt-1 mRNA in Human Renal Biopsy Samples
To confirm renal production of sFlt-1 and assess the effect of renal dysfunction, we developed a specific quantitative real-time polymerase chain reaction system to measure the levels of sFlt-1 mRNA in human renal biopsy specimens (online-only Data Supplement Figures IIIA and IIIB). Notably, not only was sFlt-1 mRNA present in the biopsied samples, but its level also had a significantly positive correlation with eGFR (r=0.25, P=0.032; Figure 1B).

Coronary Artery Disease Worsens With Progression of Renal Dysfunction
The number of coronary arteries with >75% stenosis was significantly different according to renal dysfunction (P=0.007; online-only Data Supplement Figure IC). Scores obtained by Gensini’s method for evaluating global coronary

coronary atherosclerosis were also significantly different according to renal dysfunction (P=0.015; online-only Data Supplement Figure ID).

Relationship Between PIGF/sFlt-1 Ratio and Coronary Artery Disease
Levels of sFlt-1 in plasma collected from the aorta tended to be lower (P=0.0656; online-only Data Supplement Figure IE), and there was a corresponding tendency for PIGF to be higher as the number of diseased vessels increased (P=0.0513; online-only Data Supplement Figure IF). In contrast, plasma PIGF levels were unaffected by differences in renal function (online-only Data Supplement Figure IIA); consequently, the PIGF/sFlt-1 ratio had a significantly negative correlation with eGFR (r=−0.27, P<0.001; Figure 1C).

Although plasma levels of sFlt-1 and PIGF did not differ significantly, the PIGF/sFlt-1 ratio was significantly different according to the number of coronary arteries that showed >75% stenosis (PIGF/sFlt-1 ratio in patients with 0-, 1-, 2-, and 3-vessel disease: 0.07±0.01, 0.08±0.01, 0.09±0.01, and 0.11±0.01, respectively; P=0.023; Figure 1D).

Experimental Study
Effects of 5/6 Nephrectomy on Physical and Biochemical Parameters in ApoE-Deficient Mice
The 5/6 nephrectomy procedure had no significant effects on body weight, blood pressure, heart rate, or lung/body weight ratio compared with control apoE-deficient mice (Table 3). In contrast, heart/body weight ratios at the end of the study were significantly higher in 5/6-nephrectomized mice than in control mice, and serum urea and creatinine levels were also significantly higher. Total serum cholesterol and triglyceride levels were also higher in the 5/6-nephrectomized mice.

Serum sFlt-1 concentration was lower in the 5/6-nephrectomized mice (11.45±0.44 versus 13.24±0.39 ng/mL, P=0.001; Figure 2A), whereas serum PIGF-2 concentration was higher in those mice than in control mice (20.4±3.8 versus 10.2±2.1 pg/mL, P=0.01; Figure 2C), which made the PIGF-2/sFlt-1 ratio significantly higher in 5/6-nephrectomized mice than in control mice (0.86±0.22 to 1.75±0.30).

Table 3. Hemodynamics and Biological Parameters in Control and 5/6-Nephrectomized ApoE-Deficient Mice at the End of the Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control Mice</th>
<th>5/6-Nephrectomized Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS (n=10)</td>
<td>sFlt-1 (n=10)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26.0±0.6</td>
<td>26.0±0.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>87±2.7</td>
<td>88±3.4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>50±2.1</td>
<td>52±1.4</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>658±16</td>
<td>650±27</td>
</tr>
<tr>
<td>Heart/body weight ratio (mg/g)</td>
<td>4.05±0.07</td>
<td>4.18±0.09</td>
</tr>
<tr>
<td>Lung/body weight ratio (mg/g)</td>
<td>5.38±0.07</td>
<td>5.41±0.12</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>28.6±1.2</td>
<td>35.1±4.1</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.70±0.08</td>
<td>1.12±0.13</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>2242±122</td>
<td>2176±147</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>116.7±8.5</td>
<td>160.9±29.6</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
*P<0.05, †P<0.01, ‡P<0.001 vs control PBS.
The effect of 5/6 nephrectomy (5/6NR) on serum sFlt-1 and PlGF-2 levels and renal expression of sFlt-1 mRNA in apoE-deficient mice. A and B, Both serum sFlt-1 levels (A) and renal sFlt-1 mRNA expression (B) were significantly lower in 5/6-nephrectomized apoE-deficient mice than in control apoE-deficient mice. C, Serum PlGF-2 levels were significantly higher in 5/6-nephrectomized apoE-deficient mice than in control apoE-deficient mice. D, PlGF-2/sFlt-1 ratios were also higher in 5/6-nephrectomized apoE-deficient mice than in control mice. **P<0.01. Data are mean±SEM.

1.86±(0.38 ×10⁻³; P<0.01; Figure 2D). Renal expression of sFlt-1 mRNA was significantly lower in 5/6-nephrectomized mice (1.38±0.13 versus 0.73±0.11 arbitrary units normalized to GAPDH mRNA; P<0.01; Figure 2B). Serum VEGF concentrations were similar in 5/6-nephrectomized and control apoE-deficient mice (118.2±4.7 versus 138.0±7.7 pg/mL, P=0.062).

5/6 Nephrectomy Aggravates Atherosclerosis and Macrophage Infiltration in ApoE-Deficient Mice

As shown in Figure 3, the relative plaque areas in the thoracoabdominal aorta and aortic root were significantly larger in 5/6-nephrectomized apoE-deficient mice treated with PBS than in control apoE-deficient mice (thoracoabdominal aorta: 27.3±1.1% versus 15.2±0.9%, P<0.001; aortic root: 48.8±2.0% versus 41.9±1.4%, P=0.014). Moreover, Figure 4 shows that there was significantly greater macrophage infiltration into the atherosclerotic plaque of the aortic root in 5/6-nephrectomized mice than in control mice (33.3±1.8% versus 21.4±1.6%, P<0.001).

Effect of rhsFlt-1 Administration in ApoE-Deficient Mice

Repetitive intraperitoneal administrations of rhsFlt-1 had no effect on hemodynamics or biochemical parameters in apoE-deficient mice, as summarized in Table 3. Endogenous renal expression of sFlt-1 mRNA did not change in either the 5/6-nephrectomy or the control group.

Administration of rhsFlt-1 to control apoE-deficient mice had no significant effect on atherosclerotic plaque area compared with mice administered PBS (thoracoabdominal aorta: 13.0±0.8% versus 15.2±2.4%, P=0.187; aortic root: 45.7±2.0% versus 41.9±1.4%, P=0.185). However, replacement treatment with repeated injections of rhsFlt-1 to 5/6-nephrectomized apoE-deficient mice reduced the progression of atherosclerosis compared with 5/6-nephrectomized mice administered PBS (thoracoabdominal aorta: 21.6±1.4% versus 27.3±1.1%, P<0.001; aortic root: 41.8±2.0% versus 48.8±2.0%, P<0.01; Figure 3). In 5/6-nephrectomized mice, repeated rhsFlt-1 administration significantly reduced macrophage infiltration into atherosclerotic plaques compared with controls (28.4±2.1% versus 33.3±1.8%, P=0.024; Figure 4), although in control apoE-deficient mice, macrophage infiltration into atherosclerotic plaques did not differ between mice administered rhsFlt-1 and those administered PBS (17.8±1.7% versus 21.4±1.6%, P=0.205).

Discussion

A worsening of atherosclerosis commonly accompanies renal dysfunction, but the underlying molecular mechanism is not yet fully understood. The present study demonstrates that renal production of sFlt-1 and the corresponding plasma levels of the peptide decline with progression of renal dysfunction in both clinical and experimental settings. Furthermore, administration of rhsFlt-1 inhibits renal dysfunction–induced exacerbation of atherosclerosis in an apoE-deficient mouse model. Given that sFlt-1 is an endogenous antagonist of PlGF, a proatherogenic factor, the present findings suggest that the decline of circulating sFlt-1 in patients with renal dysfunction may play a significant role in the worsening of their atherosclerosis.

sFlt-1 contains the extracellular ligand-binding domain of the full-length, membrane-spanning Flt-1 receptor and is generated by alternative splicing of the same pre-mRNA that
encodes Flt-1. Fli-1 mRNA is reportedly expressed in vascular endothelial cells in the lung, heart, kidney, and brain, as well as in placental trophoblasts, monocytes/macrophages, and renal mesangial cells, especially in patients with mesangial proliferative glomerulonephritis. However, studies of the expression profile of sFlt-1 mRNA under normal and pathophysiological conditions are lacking. In the present study, we confirmed that sFlt-1 mRNA is expressed in the kidney. Furthermore, we showed that there is a significant step-up in plasma sFlt-1 levels between the renal vein and aorta and that plasma sFlt-1 levels in the renal vein correlate significantly with those in the aorta. This suggests that renal sFlt-1 production makes a considerable contribution to the levels of peripheral circulating sFlt-1. In patients with renal dysfunction, both renal sFlt-1 production and levels of circulating sFlt-1 decline with progression of renal dysfunction. Moreover, these reductions in renal expression of sFlt-1 mRNA and circulating sFlt-1 were confirmed in a mouse model of chronic renal dysfunction.

To interpret the effects of reduced plasma levels of sFlt-1 in renal dysfunction, it is necessary to consider the relationship between sFlt-1 and its ligand, PlGF. Recent studies have shown that in addition to its angiogenic effects, PlGF exerts such proatherogenic effects as recruitment and adhesion of monocytes, production of proteolytic factors, induction of thrombus formation by stimulation of tissue factor secretion, and plaque destabilization. Because sFlt-1 acts as a natural PI GF antagonist, we presume that when the circulating sFlt-1/PI GF ratio is reduced, the action of PI GF is augmented. In the peripheral circulation, free PI GF, free sFlt-1, and the PI GF–sFlt-1 complex are present simultaneously. Although it is not yet clear which of these most closely reflects PI GF–Flt-1–mediated proatherogenic activity, and it is not clear how the assay system for PI GF or sFlt-1 cross-reacts with the PI GF–sFlt-1 complex, the present study suggests that the PI GF/sFlt-1 ratio is most closely correlated with the severity of atherosclerosis in both patients with renal dysfunction and 5/6-nephrectomized apoE-deficient mice. Although further study is needed to elucidate the clinical significance of the PI GF/sFlt-1 ratio, we hypothesized that reduced plasma sFlt-1 levels are at least associated with a relative increase in PI GF–Flt-1–mediated proatherogenic signaling in renal dysfunction.

To test this idea, we investigated whether exogenous administration of rhsFlt-1 would reverse the exacerbation of atherosclerosis caused by 5/6 nephrectomy in apoE-deficient mice. We found that repeated intraperitoneal administration of rhsFlt-1 significantly reduced plaque area and infiltration of plaques by macrophages in the aortas of 5/6-nephrectomized apoE-deficient mice. These findings are consistent with previous studies showing that local adenoviral PI GF-2 delivery promotes atherogenic neointima formation in hypercholesterolemic rabbits, that atherogenic effects are attenuated in apoE and PI GF double-knockout mice, and that an antibody against Flt-1 reduces atherosclerotic plaque growth and vulnerability. We also found that serum indices of uremia and lipid concentrations were higher in 5/6-nephrectomized apoE-deficient mice than in control mice, which is consistent with previous findings. Administration of sFlt-1 reduced atherosclerotic plaque formation without affecting serum urea or lipid levels, but it did significantly reduce infiltration of macrophages into aortic tissues. We therefore suggest that a reduction in circulating sFlt-1 levels in 5/6-nephrectomized apoE-deficient mice worsens atherosclerosis by enhancing the inflammatory processes related to increases in PI GF–Flt-1 signaling. This notion is supported by our previous findings that PI GF is rapidly expressed in myocardial infarct tissue during the acute phase of myocardial infarction and that elevation of plasma PI GF levels stimulates infiltration of monocytes into the myocardium.

Atherosclerosis is more commonly observed in the elder population than in the younger population. In the present study, we adopted the equation for glomerular filtration rate, which is a function of serum creatinine level and age, to investigate the influence of renal dysfunction on the plasma sFlt-1 level. Although there was no significant correlation between plasma level of sFlt-1 and age, it remains to be determined whether other unknown factors, or patients’ backgrounds, that would be related to aging or decreased renal function could influence the reduction of circulating sFlt-1 in patients with renal dysfunction.

According to all findings in the present study, we propose the possibility that sFlt-1 plays at least a partial role in the cause-and-effect relationship between renal dysfunction and the worsening of atherosclerosis. Of course, unknown factors related to renal dysfunction or aging, such as enhanced activity of the renin-angiotensin-aldosterone system, en-
hanced superoxide production, and accumulation of an endogenous inhibitor of nitric oxide synthesis, could be involved in the mechanism for worsening of atherosclerosis in renal dysfunction. Further studies are necessary to better understand the complex mechanism for this. sFlt-1 has also been studied extensively in the field of obstetrics. In patients with preeclampsia, serum sFlt-1 is elevated to abnormally high levels, and Maynard et al showed that such preeclamptic symptoms as hypertension and renal dysfunction could be induced by administration of an adenoviral vector harboring sFlt-1. In the present study, however, administration of rhsFlt-1 did not affect either blood pressure or renal function. This most likely reflects the lower concentrations of sFlt-1 seen after treatment in the present study. In the present study, serum sFlt-1 levels were orders of magnitude lower than in the study by Maynard et al. sFlt-1 also antagonizes VEGF, in part by interrupting signal transduction via Flt-1 and Flk-1. It is thus possible that VEGF is involved in the exacerbation of atherosclerosis in patients with renal dysfunction and in S/6-nephrectomized apoe-deficient mice. In the present clinical study, however, serum VEGF levels did not differ in accordance with renal dysfunction (online-only Data Supplement Figure IIB), and the VEGF/sFlt-1 ratio did not vary as a function of the severity of coronary atherosclerosis (online-only Data Supplement Figure IIF). In the context of the present study, it is therefore unlikely that coronary atherosclerosis was brought about through a relative increase in VEGF.

In conclusion, the present study indicates that a reduction in circulating levels of sFlt-1 in renal dysfunction is associated with the worsening of atherosclerosis.

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Disclosures
None.

References


CLINICAL PERSPECTIVE

Chronic kidney disease is a worldwide public health problem not only because it leads to end-stage renal failure but also because it is an independent risk factor for atherosclerosis-related cardiovascular events. Accumulating evidence indicates atherosclerosis is usually worsened in patients with renal dysfunction, and the risk of cardiovascular disease increases sharply as the estimated glomerular filtration rate declines. Additionally, more than 50% of deaths among patients with end-stage renal failure are due to cardiovascular events. Although it is clear that most cardiovascular events in renal dysfunction result from atherosclerosis, the underlying molecular mechanism responsible for the worsening of atherosclerosis in renal dysfunction is not yet fully understood. Consequently, an effective therapeutic strategy is still lacking. Here, we examine the role played by soluble fms-like tyrosine kinase-1 (sFlt-1), an endogenous antagonist of the proatherogenic cytokine placental growth factor (PIGF), in the worsening of atherosclerosis seen in patients with renal dysfunction and in an animal model of renal failure. This report describes our novel observation that circulating sFlt-1 levels are reduced in patients with renal dysfunction in proportion to the severity of the disease, whereas there is no change in plasma PIGF levels. Moreover, renatil production of sFlt-1 is also diminished in patients with renal dysfunction, and replacement treatment with recombinant human sFlt-1 reduces fivesixths-nephrectomy–induced worsening of atherosclerosis of apolipoprotein(E)-deficient mice. Thus, the present findings provide a new insight into the molecular mechanism for worsening of atherosclerosis in renal dysfunction and could lead to a new effective therapeutic strategy.