Recombinant Vascular Endothelial Growth Factor 121 Attenuates Hypertension and Improves Kidney Damage in a Rat Model of Preeclampsia

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Abstract—Inhibitors of angiogenic factors are known to be upregulated, and their levels increase in the maternal circulation before the onset of preeclampsia. We reproduced a previously characterized model of preeclampsia by adenoviral overexpression of the soluble vascular endothelial growth factor (VEGF) receptor sFlt-1 (also referred to as sVEGFR-1) in pregnant and nonpregnant Sprague-Dawley rats. Animals were treated with VEGF121 at 0, 100, 200, or 400 μg/kg once or twice daily (n=8 per group; 64 total) and compared with normal control animals (n=4 per group) by examination of systolic blood pressure, urinary albumin and creatinine, renal histopathology, and glomerular gene expression profiling. sFlt-1 expression induced hypertension with proteinuria and glomerular endotheliosis and significant changes in gene expression. VEGF121 treatment alleviated these symptoms and reversed 125 of 268 sFlt-1–induced changes in gene expression. VEGF121 had beneficial effects in this rat model of preeclampsia without apparent harm to the fetus. Further study of VEGF121 as a potential therapeutic agent for preeclampsia is warranted. **(*Hypertension*. 2007;50:686-692.)**

Key Words: VEGF121 ■ preeclampsia ■ sFlt-1 ■ blood pressure ■ glomerular endotheliosis ■ sVEGFR-1

Preeclampsia is a pregnancy-specific syndrome characterized by the onset of hypertension and proteinuria after 20 weeks of gestation, which affects ≈5% of all human pregnancies and remains a leading cause of maternal and fetal morbidity and mortality.[1,2] Recent data have suggested a crucial role for a soluble vascular endothelial growth factor receptor, sFlt-1 (also known as sVEGFR-1) in the pathogenesis of preeclampsia. sFlt-1 acts by binding both VEGF-A and placental growth factor and, thus, inhibiting VEGF/placental growth factor signaling in the vasculature. Moreover, sFlt-1 can also form inactive heterodimers with VEGFR-2. Levels of sFlt-1 are elevated 3- to 4-fold in women who have been diagnosed with the disease[3] and begin to climb above levels in normal pregnancies within 5 weeks before diagnosis. In addition, overexpression of sFlt-1 in pregnant rats gave rise to elevations in blood pressure, proteinuria, and renal histological lesions that resemble human preeclampsia (glomerular endotheliosis).[4] This has led to the hypothesis that preeclampsia may be a disease of VEGF deficiency brought about by the overabundance of a VEGF antagonist.

VEGF-A is a homodimeric member of the platelet-derived growth factor class of Cys-knot growth factors that acts on the vascular endothelium[5] to regulate vascular tone and contributes to vascular health by suppression of endothelial apoptosis, inhibition of leukocyte adhesion, and inhibition of platelet aggregation and thrombosis.[6] Several splice variants of VEGF have been characterized; however only the 121- and, to a lesser degree, the 165-amino acid forms are efficiently secreted and found in circulation.[7] The biological role of VEGF depends on its interaction with 2 signaling receptors, Flt-1 (VEGFR-1) and VEGFR-2. Deprivation of VEGF activity induced by overexpression of sFlt-1 in rats[4] or anti-VEGF antibodies in cancer chemotherapy[8] causes hypertension and proteinuria, suggesting that VEGF activity is essential for maintaining homeostasis of the kidney glomerulus.[4] The critical role of VEGF for the maintenance of the normal glomerular function has been further demonstrated through the deletion of a single allele of VEGF in the glomerular podocytes, which results in glomerular endotheliosis resembling human preeclampsia.[9]

We, therefore, hypothesized that agents that bind sFlt-1, such as VEGF, may be useful therapeutic agent in preeclampsia. In the present study we demonstrate the efficacy of VEGF121 therapy in a pregnant rat model of preeclampsia, characterized by overexpression of sFlt-1, and characterize the dose-response relationship and effects of dose timing.

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Finally, we show that the effects of VEGF121 on blood pressure are more pronounced in the setting of hypertension.

Methods

Reagents

The recombinant adenovirus expressing murine sFlt(1-3) has been described previously[10] and was amplified at a commercial facility (Qbiogene). VEGF121 used in these studies was from protein expressed in *Escherichia coli* and formulated in 8 mmol/L of citrate (pH 4.5). Fermentation and purification were performed in the process development laboratories at Scios, Inc.

Animal Model and VEGF121 Treatment

All of the animal experiments were performed under protocols that followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee at Scios.

Pregnant Rats

Pregnant Sprague-Dawley rats (Harlan) were injected intravenously into the tail vein on day 8 of pregnancy (early second trimester) with either Adv-sFlt-1 at a dose of 9×10^{11} viral particles (VP)/kg (or 6×10^{10} plaque-forming units per kilogram; n=41), the same dose of control virus lacking sFlt-1 (n=4), or PBS for animals in the normal control group (n=4). Plasma sFlt-1 levels were measured by mouse sFlt-1 ELISA kits (MVR100, R&D Systems) 72 hours after the adenoviral injection. Rats infected with sFlt-1 adenovirus were selected for study (n=16) based on plasma sFlt-1 level (121 to 6637 ng/mL) and treated with either 400 μg/kg of VEGF121 (n=8) or vehicle (n=8) twice a day subcutaneously for 6 days.

Nonpregnant Rats

Adv-sFlt-1 (6×10^{11} VP/kg or 4×10^{9} plaque-forming units per kilogram), an equal titer of adenovirus lacking sFlt-1, or PBS as a normal control was injected intravenously via the tail vein of Sprague-Dawley rats. Plasma sFlt-1 levels were measured 48 hours after the adenoviral injection. Animals transfected with sFlt-1 were selected for study based on plasma sFlt-1 levels (50 to 1000 ng/mL) and treated either twice daily or once daily for 6 days with VEGF121 SC at 400 μg/kg (n=8 for each dose regimen), 200 μg/kg (n=8 for each dose regimen), 100 μg/kg (n=9 for the BID group and n=8 for the QD group), or vehicle (n=8 for each dose regimen) and compared with normal control (n=4 for each dose regimen).

For the final study day, rats were housed in metabolic cages for urine sample collection. The last dose of VEGF121 was given 2 hours before blood pressure measurement. Systolic blood pressure (SBP) was measured by the tail-cuff technique and recorded by an IITC blood pressure recording system before the termination of the experiment. Rats were restrained in the testing chamber (28°C to 32°C) for 10 minutes before recording pressure data.

Determination of Plasma sFlt-1, Urinary Albumin, and Creatinine

At the end of the treatment period, rats were euthanized by CO_2 asphyxiation, and blood from the right ventricle was collected in EDTA-containing tubes. Plasma sFlt-1 levels were determined using ELISA kits obtained commercially (MVR100, R&D Systems). Urinary albumin was measured by ELISA kits (Nephrat II, Exocell), and urinary creatinine was measured by the alkaline picrate method (Exocell).

Measurement of VEGF121 in Plasma

VEGF121 was measured using a protocol that was substantially free of interference by the presence of circulating sFlt-1. Briefly, a monoclonal anti-VEGF antibody, 5.1 (Scios Inc), was absorbed to a Fluoro Nunc 96-well plate (Nalge Nunc International). After applying a plasma sample or VEGF121 standard, the plate was washed and blocked with a solution of 1% BSA in PBS. To prevent interference by sFlt-1, plates were treated with 12 μL of 5% triflouroacetic acid for 30 minutes followed by neutralization with 7.5 μL of 1 N sodium hydroxide. A goat anti-human polyclonal antibody conjugated with horseradish peroxidase was added to detect immunoreaction. Absorbance of the sample wells was quantified relative to the standard curve in the range of 0.73 to 3000 pg/mL.

Histopathology

Kidneys were bisected longitudinally through the pelvis. Half of the kidney was fixed in 10% buffered formalin for 72 hours and processed for paraffin embedding and sectioning. The other half of the kidney was prepared for frozen sectioning, and hematoxylin & eosin staining was performed. These frozen sections were used for laser capture microdissection-coupled cDNA microarray and real-time RT-PCR. A series of 4-μm sections was cut and processed for periodic acid Schiff and hematoxylin & eosin stains on the formalin-fixed tissue. Kidney damage was analyzed semiquantitatively in 100 randomly selected glomeruli per kidney based on histopathologic changes, including endothelial swelling, occlusion of capillary loops, and protein resorption droplets. Grades from 0 to 4 were given based on the severity of the glomerular lesions as follows: (1) grade 0: normal; (2) grade 1: mild capillary loop occlusion, no protein droplets; (3) grade 2: moderate capillary loop occlusion, <25% having protein droplets; (4) grade 3: severe capillary loop occlusion with <50% having protein droplets; and (5) grade 4: very severe capillary loop occlusion with >50% having protein droplets. A glomerular lesion index was calculated from the sum of the individual scores averaged across animals in a study group.

Laser Capture Microdissection

Kidney tissue was embedded in OCT, and a series of 7-μm–thick frozen sections was obtained for HEAMCen (STHEM30, American Master Tech) staining. Forty glomeruli (≈3000 cells) in the cortex of the kidney were randomly captured using an AutoPix automated laser capture microdissection system (Molecular Devices Corporation) and preserved in XB buffer (03k249, Molecular Devices Corporation) for mRNA isolation.

cDNA Microarray and Real-Time RT-PCR

Gene expression profiles were determined from cDNA microarrays containing 8600 elements derived from clones isolated from normalized cDNA libraries or purchased from ResGen (Invitrogen) as described previously.[11] Differential expression values were expressed as the ratio of the median of background-subtracted fluorescent intensity of the experimental RNA to the median of background-subtracted fluorescent intensity of the control RNA. RNA was isolated on day 8 after adenoviral injection from ~40 harvested glomeruli from rats overexpressing sFlt-1s (N=3), from rats overexpressing sFlt-1 that were treated with VEGF121 (400 μg/kg, twice daily; N=3), or from rats treated with control virus lacking sFlt-1 (N=3). The hierarchical clustering algorithm contained in Spotfire software was used for functional clustering analysis.

Real-time RT-PCR confirmation of the microarray results was performed for 6 affected genes, *plasminogen activator inhibitor-1 (PAI-1), osteopontin, matrix metalloproteinase-9 (MMP-9), matrix metalloproteinase-12 (MMP-12), insulin-like growth factor binding protein (IGFBP5),* and *chemokine C-X-C motif ligand 10 (IP-10).* Results were normalized against 18S rRNA. Real-time RT-PCR was performed using a Prism 7900 Sequence Detection System (Applied Biosystems). Relative quantitation of gene expression was calculated using the comparative threshold cycle number for each sample fitted to a 5-point standard as described previously.[11] Sequence-specific primers and probes were designed using Primer Express version 2 software (Applied Biosystems). Sequences of primers and probes can be found in Supplementary Table S1 (available at http://hyper.ahajournals.org).

Implantation of Telemetric Device

Implantation of the radiotelemetric transmitters (DSI/Transoma) was performed according to the procedure described previously

by Brockway et al[12] Under general isoflurane anesthesia and aseptic conditions, a midline abdominal incision was made, the skin and abdominal muscles were retracted, and then the lower part of the descending abdominal aorta was carefully exposed and dissected with fine forceps. After temporary clamping of the aorta, the tip of the catheter/sensor was inserted in the lumen of the aorta (with the tip against the flow and just inferior to the renal arteries) and then fixed in place by tissue adhesive (3 mol/L of Vetbond) and a sterile patch of paper fiber. The body of the radiotelemetric transmitter was immobilized in the peritoneal cavity by suturing it to the ventral abdominal musculature at the incision site, and the wounds and skin were successively sutured. The animals were allowed to recover for ≥5 days before further study procedures.

Telemetric Data Acquisition and Analysis

A Physiotel radiotelemetric transmitter (TL11M2-C50PXT) was used for measurement and transmission of signals of SBP and diastolic blood pressure, mean arterial blood pressure, and heart rate of rats. A radioreceiver platform or radar (RPC-1 receiver) was placed under the cage of each animal to receive radiofrequency signals transmitted by the electronic device. A Data Exchange Matrix (RMX-1) was used to multiplex multiple cage signals to the computer, and a PC-based data acquisition system (Dataquest ART Gold System version 2.0) was used for data collection and offline analysis of hemodynamic signals.

Measurement of Acute Hemodynamic Effects of VEGF121

Normotensive rats or rats made hypertensive by infection, 5 days previously, with Adv-sFlt-1 were cannulated with a PE50 catheter into the jugular vein for later administration of treatments. After a further day of recuperation, baseline measurements were made, and rats received VEGF121 infusion at rates indicated in the text for 180 minutes. Hemodynamic measurements were made at the end of the treatment period.

Statistical Methods

All of the blood pressure and histology scores were analyzed by 1-way ANOVA followed by Bonferroni multiple-group comparison test (Instat V3.0, GraphPad). Proteinuria values were analyzed by Wilcoxon rank sum test. $P<0.05$ was accepted as statistically significant.

Results

Efficacy of VEGF121 in a Pregnant Rat Model of Preeclampsia

Overexpression of sFlt-1 using adenoviral vectors resulted in mean levels of sFlt-1 of 3 μg/mL at day 3 increasing to 12.9 μg/mL at day 9 after infection. These levels are much higher than those reported previously for preeclamptic patients[4,13]; however, recent assay refinements have led to much higher estimates.[14] Overexpression of sFlt-1 resulted in significantly increased SBP (178±15 versus 126±15 mm Hg; $P<0.001$; Figure 1A) and an increase in proteinuria as measured by the urine albumin:creatinine ratio (7555 versus 50 μg/mg, Figure 1B) compared with control rats. Treatment of pregnant sFlt-1–infected rats with VEGF121 at 400 μg/kg twice a day for 6 days significantly alleviated elevated SBP as compared with vehicle treated rats (144±13 versus 178±15 mm Hg; $P<0.001$; Figure 1A). Likewise, there was reduction in proteinuria (72% decrease in urine albumin:creatinine ratio; Figure 1B) resulting from VEGF treatment. Kidney damage induced by sFlt-1 overexpression was characterized by glomerular enlargement, endothelial cell swelling, occlusion of the capillary loops (endotheliosis), and accumulation of protein resorption droplets. Adv-sFlt-1–infected rats treated with VEGF121 showed significant improvement in glomerular histology with improvements in capillary patency, reductions in protein deposits, and endothelial swelling (Figure 2). When the glomerular histological lesions were quantitated as described in the Methods section, Adv-sFlt-1–infected rats treated with VEGF121 gave a significant reduction in average histological score (180 versus 66; $P<0.05$).

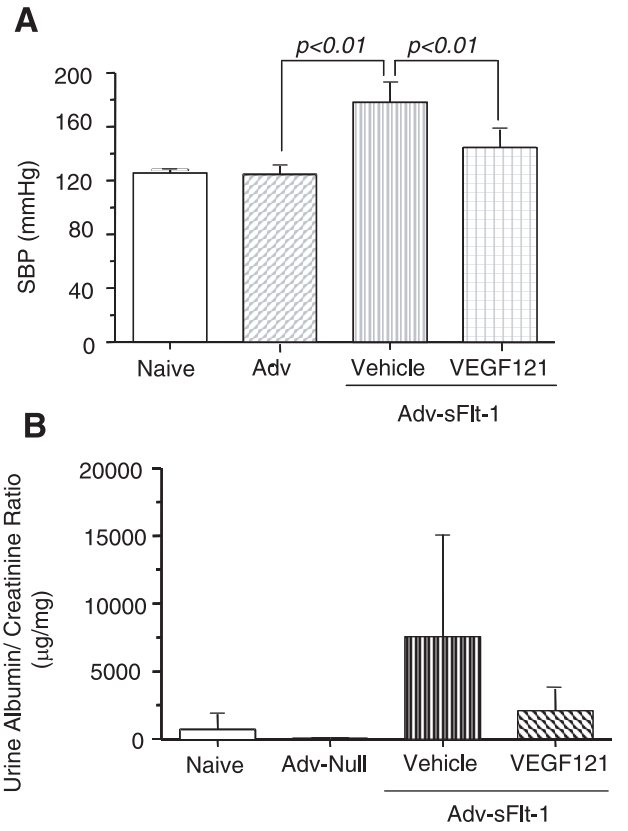

Figure 1. Effect of VEGF121 on SBP (A) and proteinuria (B) induced by overexpression of sFlt-1 in pregnant rats. Rats uninfected (Naive), infected with null virus (Adv-Null), or Adv-sFlt-1 were treated as indicated with vehicle or 400 μg/kg BID for 6 days. Data are presented as mean±SD. Data represent N=4 each for the Naive and the Adv-Null groups and N=8 each for the sFlt-1 groups.

VEGF121 treatment had no effect on fetal or placental weight (see Figure S1). Placental histology was normal (see Figure S2), and the number of resorption sites, indicative of spontaneous abortion, was unaffected by VEGF121 treatment.

Dose Ranging and Timing Studies

Dose-ranging studies were performed on nonpregnant rats to determine the minimum effective dose and dose timing of VEGF121 in the sFlt-1 model. The dose of Adv-sFlt-1 required for nonpregnant animals was somewhat reduced compared with pregnant rats (6×10^{11} versus 9×10^{11} VP/kg) perhaps because of the presence in pregnant animals of placental growth factor, which also binds to sFlt-1. Eight days after infection with Adv-sFlt-1, animals treated for 6 days with vehicle alone showed elevation of SBP to

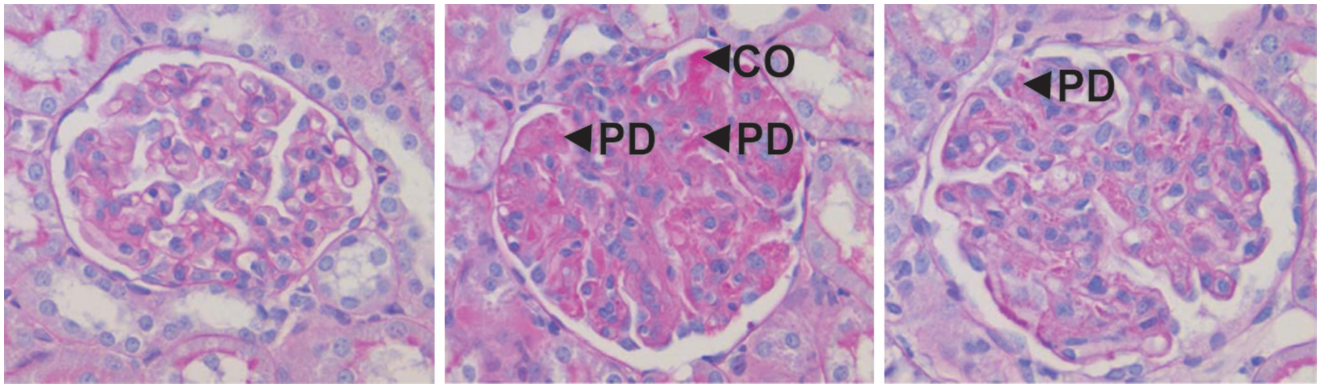

Figure 2. Improvement in glomerular histology of sFlt-1 overexpressing rats after treatment with VEGF121. Representative periodic acid Schiff's staining of rat kidneys infected with a control virus (left), sFlt-1 overexpressing virus treated with vehicle (middle), or sFlt-1 overexpressing virus treated with VEGF121 400 μg/kg BID (right). CO represents capillary occlusion and PD represents protein resorption droplets, which are both hallmarks of glomerular endotheliosis. All of the photomicrographs were taken at ×60 (original magnification).

147 mm Hg compared with 105 mm Hg in animals infected with control virus. Twice-daily treatment with 100, 200, or 400 μg/kg of VEGF121 resulted in significant reduction of SBP in all cases (120±9, 120±5, and 110±8 mm Hg respectively; $P<0.01$ compared with vehicle group; Figure 3), with only small differences between treatment groups. By contrast, Adv-sFlt-1–infected rats treated once daily with VEGF121 showed progressive, dose dependant reductions in SBP to 140±4, 123±15, and 114±5 mm Hg ($P<0.001$ in VEGF121 at 200 μg/kg and 400 μg/kg compared with vehicle group; Figure 4).

It is important to note that, in these chronic studies, no acute effect of VEGF121 on blood pressure was noted. In separate experiments where an interim blood pressure measurement was made after only 2 days of twice-daily VEGF121 dosing (400 μg/kg), the effect on blood pressure was greatly reduced and statistically insignificant in the interim result (data not shown), whereas the 6-day treatment result was highly significant (129 mm Hg versus 165 mm Hg; $P<0.001$). Thus, the effect of VEGF121 at these doses delivered by chronic subcutaneous injection does not seem to operate by an acute mechanism, perhaps requiring an extended treatment period for an effect on endothelial damage.

The differences in once- versus twice-daily dosing on sFlt-1–induced proteinuria were similar to those observed on blood pressure. VEGF121 treatment at 100, 200, and 400 μg/kg BID reduced the ratio of urinary albumin to creatinine by 77%, 95%, and 95%, respectively, compared with vehicle group (Figure 3). Once-daily treatment with VEGF121 at 100, 200, and 400 μg/kg reduced the urine albumin:creatinine ratio by 13%, 73%, and 87%, respectively ,compared with the vehicle group (Figure 4). In summary, the 100 μg/kg BID and 200 μg/kg QD doses both seem to be the threshold doses for the nonpregnant model. Thus, when total daily dose is considered, the above data show that roughly full effect is obtained by daily doses of 200 μg/kg per day, regardless of delivery as single or multiple subcutaneous injections.

Acute Hemodynamic Effects of VEGF121 Infusion

To determine the acute hemodynamic response to VEGF121, either normotensive rats or hypertensive rats expressing sFlt-1 were subjected to intravenous infusion with 10 μg/kg per minute of VEGF121 and effects on blood pressure and heart rate measured by means of a surgically implanted telemetric device. This gave rise to

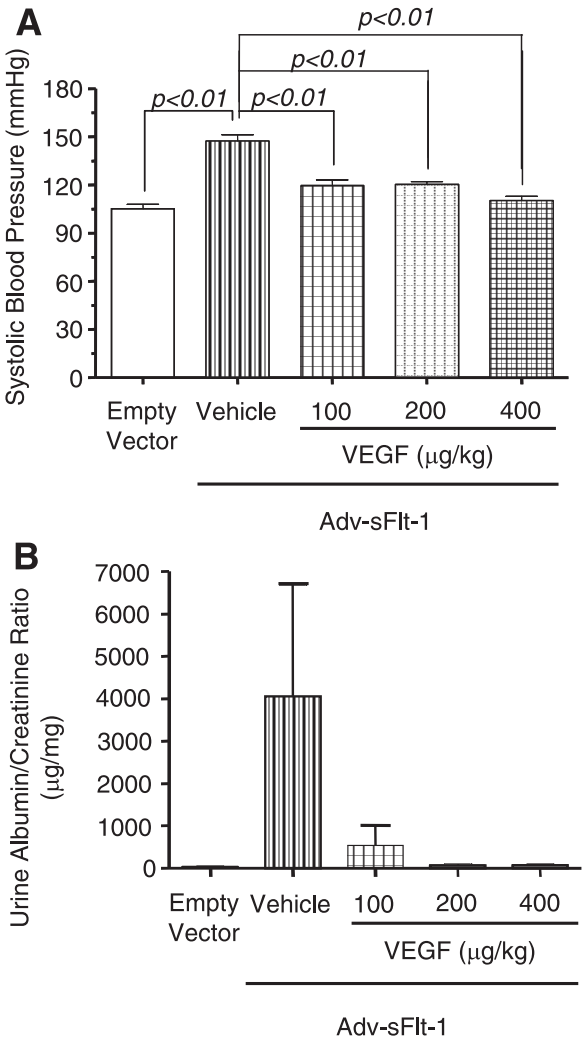

Figure 3. Effects of twice-daily doses on SBP (A) and proteinuria (B). Data represent mean±SD; n=4 in the empty vector group and n=8 in the vehicle and VEGF121-treated groups.

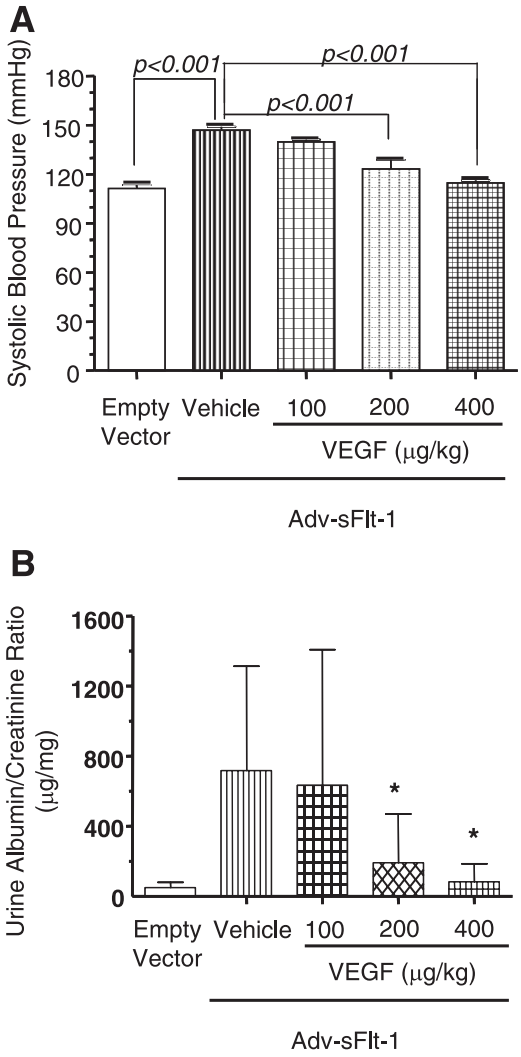

Figure 4. Effects of once-daily doses on SBP (A) and proteinuria (B). Data represent mean±SD; n=4 in the empty vector group and n=8 in the vehicle and VEGF121-treated groups. *$P<0.05$ vs vehicle group.

circulating levels, which were ≈400-fold higher than those achieved in the chronic subcutaneous studies (Table) and which were required to achieve acute hemodynamic effect. VEGF121 infusion had little effect on blood pressure in

Table 1. Pharmacokinetic Parameters for VEGF121 in Nonpregnant Rats

		Acute Telemetry Studies	
Dosing Criteria	Chronic Studies	Adv-sFlt-1	Control Virus
Route of administration	Subcutaneous	IV infusion	IV infusion
Dose	400 μg/kg	10 μg/kg-min	10 μg/kg-min
$T_{1/2}$, h	1.83	NA	NA
T_{MAX}, h	2.0	NA	NA
C_{MAX}, ng/mL	6.01	1900	3200

NA indicates data not available; $T_{1/2}$, terminal half-life; T_{MAX}, time required to attain C_{MAX}; C_{MAX}, maximal circulating concentration. For experimental details and a graphic representation of the kinetics of a 400 μg/kg SC dose, see the online supplemental data.

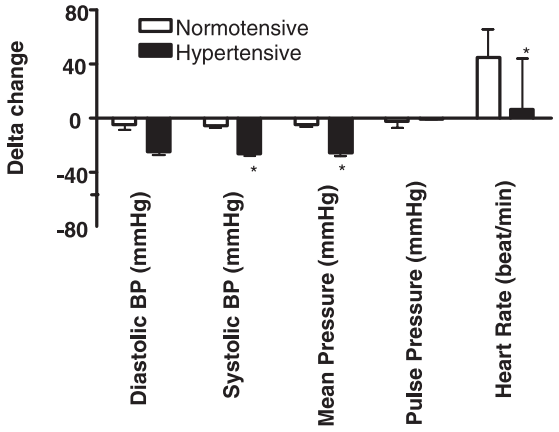

Figure 5. Comparison of hemodynamic parameters during intravenous infusion of VEGF121 into normotensive or sVEGF-R1–induced hypertensive rats. Rats infected with either control virus (n=6) or adenovirus overexpressing sFlt-1 (n=7) were infused for 3 hours with 10 μg/kg per minute of VEGF121. Hemodynamic parameters were monitored via surgically implanted telemetry devices. Measurements were made at the end of the infusion period. Data represent mean±SD. *$P<0.05$ vs normotensive animals.

normotensive rats even at these substantial infusion rates (mean reduction of 5 mm Hg; see Figure 5). By contrast, hypertensive rats experienced a substantial reduction in blood pressure (25 mm Hg in mean arterial pressure). There was an elevation in heart rate, which was significant only in normotensive animals (44.6 bpm), whereas hypertensive animals gave only a small heart rate response (6.8 bpm). Thus, there seemed to be an effect of VEGF121 on heart rate that compensated for the vasodilatory effect in normotensive animals but that was lacking in the setting of hypertension.

Effect on Gene Expression

We also examined the effect of Adv-sFlt-1 transfection and VEGF121 therapy on glomerular gene expression using cDNA microarrays. A distinct pattern of gene expression associated with overexpression of sFlt-1 was identified using a hierarchical clustering algorithm (Figure 6A). This allowed the generation of functional clusters of genes related to angiogenesis, hypoxia, inflammation, and coagulation (Figure 6B). VEGF treatment significantly reversed 129 of 312 genes that were upregulated or downregulated by sFlt-1, and the overall trend was strong toward reversal of effect (Figure 6; $P<0.05$; 1.8-fold; see also Table S2). Expression levels of 6 genes encoding soluble secreted proteins affected by sFlt-1 transfection (*PAI-1, IP-10, MMP-9, MMP-12, osteopontin,* and *IGFBP5)*, were validated by real-time PCR analysis of whole kidneys. For each of these, VEGF121 significantly reversed the glomerular gene expression changes stimulated by sFlt-1 (Figure 7).

Discussion

In the present study, we investigated the effects of recombinant VEGF121 in a rat model of preeclampsia, induced by overexpression of the soluble receptor, sFlt-1. As has been demonstrated previously,[4] elevation of plasma sFlt-1 by

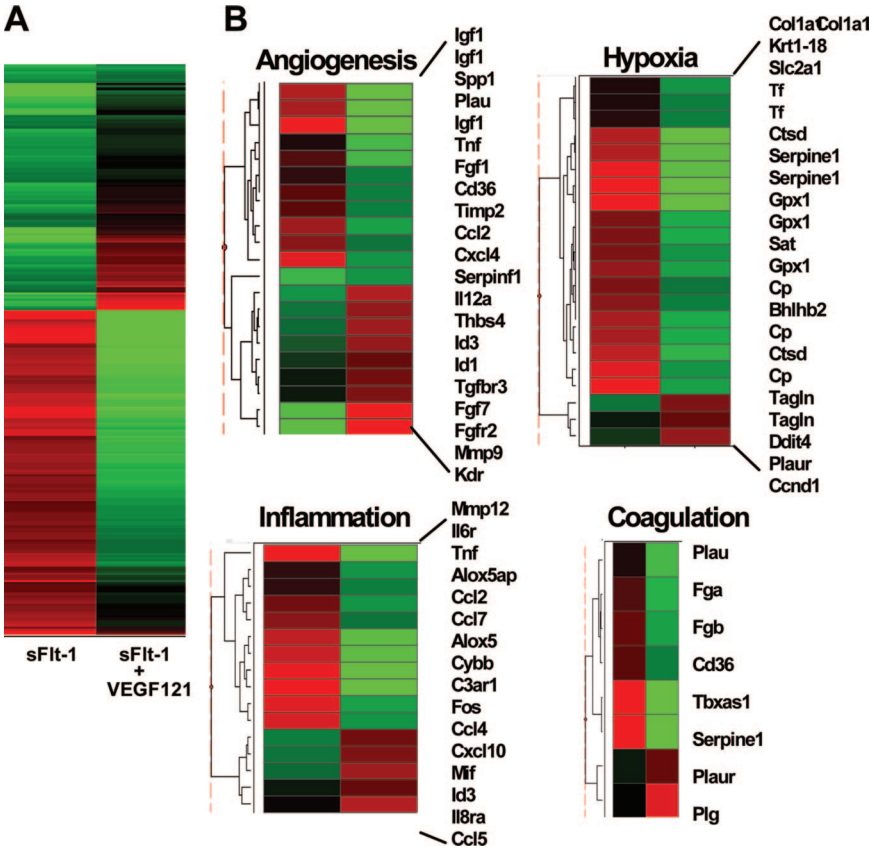

Figure 6. Glomerular gene expression patterns in response to sFlt-1 overexpression and VEGF121 treatment. Each row represents the response of a single gene to either sFlt-1 overexpression relative to control virus (left column) or VEGF121 treated sFlt-1 animals relative to untreated animals overexpressing sFlt-1 (right column). Shading represents intensity of change (red, elevated expression; green, repressed expression). A, Expression data for all of the differentially expressed genes. B, Selected data representing genes associated with angiogenesis, inflammation, hypoxia, and coagulation.

infection with Adv-sFlt-1 in rats resulted in hypertension and proteinuria resembling human preeclampsia. Histologically, kidneys from these animals show glomerular endotheliosis, reminiscent of the renal lesions traditionally ascribed to the kidney damage associated with preeclampsia in pregnant women.

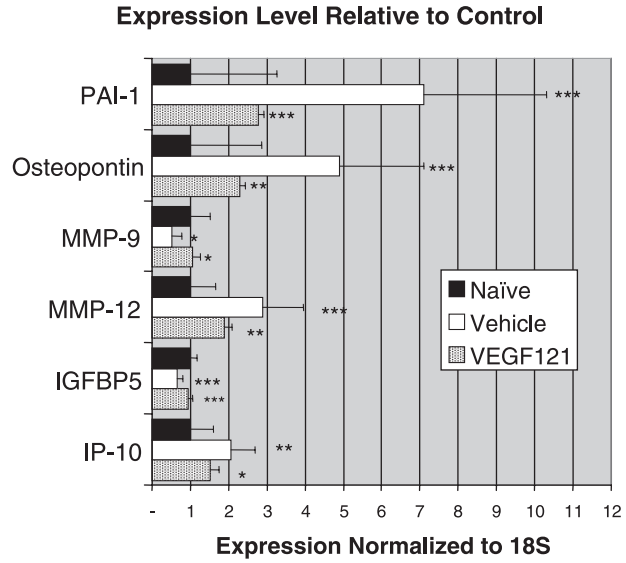

Figure 7. Validation of selected markers in whole kidney by real-time RT-PCR. Expression changes for 6 genes encoding soluble secreted proteins were determined by RT-PCR. Values are reported as mean±SD; n=8 in naive group, 10 in vehicle group, and 9 in VEGF121-treated group. Individual normalized sample values were calculated by dividing the triplicate mean value of the gene of interest with the mean triplicate values of 18s. Vehicle and VEGF121 *P* values are vs naive and vehicle groups respectively calculated by nonparametric Newman-Keuls multiple comparison test (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

Administration of recombinant VEGF121 reversed preeclamptic phenotypes, presumably by replacing natural VEGF lost to sFlt-1 antagonism. When administered twice daily, the effects were significant for reduction in SBP at all of the doses and for improvement of kidney damage at the highest dose (400 μg/kg per day). Dose dependence was more apparent in the once-daily dosing, where the highest dose (400 μg/kg) again resulted in statistically significant improvements in SBP and endotheliosis and a substantial reduction in proteinuria. When treatment regimens are compared on the basis of an equal total daily dose, there seems to be little effect of once- versus twice-daily dosing. Although the half-life of VEGF121 is relatively short (see Table 1 and Figure S3), it would seem that efficacy can be achieved without continuous exposure being maintained. Neither sFlt-1 nor VEGF121 treatment had any perceived effects on the fetus or placenta. We do not know whether therapy with VEGF121 would have any subtle long-term effects on the fetus.

Acute intravenous infusion of VEGF121 in normotensive rats had little effect on blood pressure at infusion rates up to even 50 μg/kg per minute while a substantial effect on heart rate is experienced. In hypertensive animals this pattern is reversed, with 10 μg/kg per minute giving significant reductions in blood pressure with little effect on heart rate. Similar effects were seen in earlier studies on VEGF165.[15] In these studies, which were conducted with

spontaneously hypertensive rats, both a reduced circulating level of VEGF and a baroreflex response were postulated to account for the difference between normotensive and hypertensive animals. In the current studies, circulating levels of VEGF121 are only slightly reduced in the hypertensive animals (maximal circulating concentration = 3.2 μg/mL normotensive versus 1.9 μg/mL hypertensive), eliminating this as a cause for the difference and suggesting that baroreflex response may be the operative mechanism.

We also examined the effect of Adv-sFlt-1 transfection on glomerular gene expression events that may be associated with kidney damage using cDNA microarrays. Distinct gene expression patterns were reproducibly associated with expression of sFlt-1, and VEGF121 treatment significantly reversed these effects in nearly half of the affected genes. Clustering analysis showed that a large number of genes related to angiogenesis, hypoxia, inflammation, and coagulation were affected by sFlt-1 expression and VEGF121 treatment. We used real-time RT-PCR to validate a subset of these, which represent potential soluble secreted biomarkers that may prove useful in monitoring disease progression and treatment.

It is worth noting that the circulating concentrations of sFlt-1 reported in this article are higher than what was reported in the initial publication of the sFlt-1–induced preeclampsia model.[4] These differences are likely because of differences in the methodologies used to measure sFlt-1 between previous studies and the current one.[14] It is impossible to extrapolate the effects of VEGF121 in this model to humans, because human preeclamptic pregnancies are exposed to several months of high-circulating sFlt-1 in contrast with 8 to 10 days of exposure in rats, and additional synergistic factors, such as hyperuricemia, obesity, and other circulating factors, are not addressed in this model. Studies using VEGF121 in primate models of preeclampsia that more closely resemble human preeclampsia will be needed to clarify the role of this novel therapeutic agent in preeclampsia. Although sFlt-1 overexpression seems to be crucial in determining the presence of symptoms in patients with preeclampsia, recent data have shown that a fragment of the transforming growth factor-β receptor, soluble endoglin, also has a role in modulating the severity of symptoms induced by overexpression of sFlt-1.[16] It remains unknown whether VEGF121 would also be beneficial in ameliorating the toxic effects mediated by sFlt-1 in the presence of soluble endoglin. Although not explored in this study, other agents that bind sFlt-1, such as placental growth factor or antibodies against sFlt-1, may also be promising modalities to neutralize the excess sFlt-1 in patients with preeclampsia. Studies are currently underway to determine the effect of VEGF121 in a model characterized by the overexpression of both sFlt-1 and soluble endoglin.

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Disclosures

The authors, with one exception (S.A.K.), are employees of Scios Inc. S.A.K. is a co-inventor on patents filed by the Beth Israel Deaconess Medical Center for the diagnosis and therapy of preeclampsia. S.A.K. is a consultant to Scios, Inc.

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