Pregnancy-induced hypertension (PIH) complicates 6% to 10% of pregnancies. It represents 1 of the following 4 conditions: (1) pre-existing hypertension, (2) gestational hypertension and preeclampsia; (3) pre-existing hypertension plus superimposed gestational hypertension with proteinuria; and (4) unclassifiable hypertension. Among these conditions, preeclampsia entails severe consequences for both the mother and the fetus.2,3

During the first trimester of human pregnancy, the extravillose trophoblasts must migrate and invade the maternal decidua and differentiate to facilitate the uterine spiral arterioles to undergo transformation and widen their luminal diameter, thus supplying more blood containing oxygen and nutrients to the placenta and growing fetus. One of the major contributing factors to the development of PIH is the failure of the extravillose trophoblast invasion deep into the maternal decidua.

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compromising the normal establishment of the fetomaternal circulation. Failure in the invasion process results in (1) poor perfusion of the placenta, (2) increased trophoblast apoptosis, and (3) abnormal production of antiangiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) by the placenta, which directly affect kidney function.

Recent studies have shown that altered levels of antiangiogenic factors seem to play a role in the prediction of preeclampsia. Elevated levels of sFlt1, reduced levels of placental growth factor, and an increased sFlt1: placental growth factor ratio have been reported both in women with established preeclampsia and in women before the development of preeclampsia. Also, an sFlt1: placental growth factor ratio of ≤ 38 has been proposed to predict the short-term absence of preeclampsia in women in whom the syndrome is suspected clinically. Recent clinical studies also demonstrated that deregulations in circulating sFlt1 and sEng proteins are not only present at the launch of preeclampsia but also antedate its clinical symptoms.

We recently reported that a specific placental factor named endocrine gland–derived vascular endothelial growth factor (EG-VEGF) exhibits features of potential marker of pregnancy pathologies, such as preeclampsia and intrauterine growth restriction. EG-VEGF, also called prokineticin 1, is the canonical member of a recently described family of cytokines, the prokineticin family. EG-VEGF expression is grossly restricted to endocrine tissues, including the placenta. EG-VEGF exerts its action via 2 G protein–coupled prokineticin receptors, called PROKR1 and PROKR2.

We recently showed that EG-VEGF (1) is abundantly produced during the first trimester of human pregnancy with a peak of expression just before the establishment of the fetomaternal circulation (10–12 weeks of gestation), (2) is mainly localized in the syncytiotrophoblast, (3) its expression is upregulated by hypoxia; and (4) its receptors, PROKR1 and PROKR2, are highly expressed in the placenta. Using the human placental explant model, we demonstrated that EG-VEGF is an inhibitor of extravillous trophoblast invasion. More importantly, we demonstrated that EG-VEGF circulating levels were ≥50 pg/mL in the serum of nonpregnant women and increased 5-fold during the first trimester of pregnancy (≥250 pg/mL). In relation to preeclampsia and intrauterine growth restriction, circulating EG-VEGF levels were significantly increased during the third trimester, suggesting that sustained EG-VEGF levels beyond the first trimester of pregnancy may contribute to the development of pregnancy pathologies, including PIH.

In previous studies on gravid mice, we have substantiated these findings by demonstrating that EG-VEGF is (1) expressed in the syncytiotrophoblast within the labyrinth; (2) its expression peaks during the first 11.5 days post coitus (dpc), equivalent to the first trimester in pregnant women; and (3) both its receptors are present in the placenta and exhibit similar spatiotemporal expression. This supports the use of the mouse as a model to verify the hypothesis that EG-VEGF maintenance over its normal period of production is the cause for the development of pregnancy pathologies. In this study, therefore, we aimed to enforce EG-VEGF production beyond 11.5 dpc to mimic the abnormal production observed in pregnant women. The treated gravid mice were screened for pathogenic features of the cause of PIH.

### Methods

#### Tissue Collection

All animal studies were approved by the institutional guidelines and those formulated by the European Community for the Use of Experimental Animals. Two- to 3-month old female mice were mated in the animal facility. The presence of a vaginal plug was observed at 0.5 dpc. The gravid mice were randomly assigned to receive an osmotic pump delivering either recombinant human EG-VEGF (4 μg; 0.5 μL/h for 5–7 days) or saline (Figure 1A, flowchart) at 7.5 dpc or at 11.5 dpc. The diet was maintained throughout the experimental period. Gravid mice treated at 7.5 dpc (n=20 mice) were euthanized at 12.5 dpc and those treated at 11.5 dpc (n=41 mice) were euthanized at 15.5 dpc (n=21 mice) or at 18.5 dpc (n=20 mice). The blood was drawn by cardiac puncture just before laparotomy. In another set of experiments, 20 male mice treated or not with recombinant EG-VEGF were also used. Mice were euthanized by cervical dislocation after chloral hydrate anesthesia.

#### Maternal, Fetal, and Placental Weights

Placentae and fetuses were weighed at 12.5, 15.5, and 18.5 dpc, and average weights were analyzed as raw weights. Fetal and placental weights, and the fetal to placental weight ratio, reflecting placental efficiency, were compared between the treated and the control groups.

#### Renal Histology and Function

Four-micrometer-thick sections were cut from paraffin-embedded kidney blocks, mounted on silanized slides, deparaffinized, and rehydrated. The general morphology was analyzed on selected tissue sections after staining with hematoxylin and eosin. Two pathologists (DrS Boutron and Florin) blinded to the clinical outcome evaluated 10 glomeruli from each kidney for glomerular compaction. Images were taken with an Olympus BX51F light microscope (Olympus, Rungis, France). The urine albumin to creatinine ratios were measured on 8.5, 15.5, and 18.5 dpc. Metabolic chambers were used to collect urine and feces separately. Animals were transferred to these metabolic cages at days 7.5, 14.5, and 17.5 for 24 hours.

#### Blood Pressure

The mean blood pressures were measured using a noninvasive computerized tail-cuff system (CODA 8 system, Paris, France) on 6 different days: before mating, after the plug, and at 8.5, 12.5, 15.5, and 18.5 dpc.

#### Serum EG-VEGF, sFlt1, and sEng

EG-VEGF, sFlt1, and sEng concentrations were measured in the serum of 12.5-dpc (saline, n=12; EG-VEGF treated, n=11), 15.5-dpc (n=9, saline; n=6, EG-VEGF treated), and 18.5-dpc (saline, n=4; EG-VEGF treated, n=4) gravid mice by ELISA. We used murine sFlt1 (RayBiotech, Le Perray-en-Yvelines, France), murine sEndoglin (R&D systems, Lille, France), and human EG-VEGF (PeproTech, Neuilly-sur-Seine, France) commercial assay kits. For each assay, 2 separate standard curves were constructed to allow accurate readings of samples at upper and lower ranges of the assay. All samples were in the linear range of the standard curves. The detection limit of the assay was 16 pg/mL for EG-VEGF, 100 pg/mL for sFlt1, and 62.5 pg/mL for sEng.

#### Placental Histology

Placental histology was performed as previously described. Sections were stained with hematoxylin and eosin for general histological analysis or periodic acid-Schiff for the identification of glycogen cells. Photographs were taken with a digital camera and were used to calculate the layer surface (ImageJ software) of the 3 placental zones (decidua, junctional zone, and labyrinth).

#### Immunohistochemistry of Placental Tissue

Immunohistochemistry was performed as described previously. Placental sections were incubated with the following antibodies:
anti–pan-cytokeratin (Abcam, Paris, France), anti-CD31 (DAKO, Les Ulis, France), anti–carbonic anhydrase IX (CAIX, Novus Biological), and anti–proliferating cell nuclear antigen (DAKO). Immunopositive staining was detected using a Vectastain ABC kit (Vector Laboratories, Nanterre, France) and DAB (3,3′-diaminobenzidine tetrahydrochloride) as the chromogen (Vector Laboratories).

Western Blotting of Placental Tissues
Placentae (15.5 dpc [saline, n=7; EG-VEGF treated, n=7] and 18.5 dpc [saline, n=7; EG-VEGF-treated, n=7]) were collected from different animals and processed for Western blotting analysis as previously described.17 Expression levels of PROKR1 (Covalab, Villeurbanne, France), PROKR1 (Covalab), sFlt1 (Sigma Aldrich, St. Quentin Fallavier Cedex, France), CD31, proliferating cell nuclear antigen, and anti–carbonic anhydrase IX proteins were compared in placentas from control and treated animals. β-actin was used to standardize protein loading.

Real-Time Reverse Transcriptase Polymerase Chain Reaction Analysis of Placental Tissue
Total RNAs were extracted from 15.5-dpc (saline, n=7; EG-VEGF treated, n=7) and 18.5-dpc (saline, n=7; EG-VEGF-treated, n=7) placentae, using the Nucleospin RNA kit (Macherey-Nagel). Quantitative real-time reverse transcriptase polymerase chain reaction was used as previously described.17 Expression of proliferin, Hand1, Hand2, prolactin, Mash 2, Gcm1 mRNA expression was normalized to RPL13 mRNA expression level. Sequences of the used polymerase chain reaction primers are listed in the Table.

Statistical Analysis
Statistical comparisons were made using 1-way ANOVA analysis followed by Holm–Sidak method and tested for homogeneity of variance and normality (P<0.05). Student t test was also used when appropriate. Calculations were performed using SigmaStat (Jandel Scientific Software, San Rafael, CA).

Results
Maternal Serum EG-VEGF Throughout Mouse Gestation
We compared EG-VEGF circulating levels in male mice, in nongravid female mice, and in gravid mice throughout their gestation. EG-VEGF levels were significantly different between nongravid and gravid mice at all gestational ages examined (Figure 1B). In male mice, EG-VEGF levels were ≈30 pg/mL and were slightly lower than those in female mice. EG-VEGF levels increased significantly during gestation reaching a peak ≈12.5 dpc (≈60 pg/mL). These levels decreased, thereafter, to reach levels similar to those of nongravid mice.

To induce a sustained elevation of EG-VEGF levels beyond 11.5 dpc (Figure 1A), gravid mice were implanted with osmotic pumps delivering EG-VEGF (4 μg; 0.5 μL/h for 5–7 days). The treatments started at 7.5 dpc and ended at 12.5 dpc or started at 11.5 dpc and ended at either 15.5 or 18.5 dpc. Figure 1C reports EG-VEGF serum levels in gravid mice at 12.5, 15.5, and 18.5 dpc. Mice were either saline treated or EG-VEGF treated. Data represent mean±SEM (*P<0.05).
gestational ages were >2.5×, mimicking the degree of increase observed in preeclampsia women.12

### Structural Analysis of Placental Zones at 12.5, 15.5, and 18.5

Each of the 3 placental zones reflects an aspect of its development. An increase in the size of decidua reflects a higher degree of trophoblast invasion.18,19 An increase in the junctional zone is correlated to a higher degree of placental hypoxia.20 The size of the labyrinth is informative on the degree of placental growth.21 Analysis of placentae collected at 12.5 dpc showed a significant decrease in the decidual zone in the placenta of EG-VEGF–treated dams, along with an increase in the junctional zone. No change in the labyrinth zone was observed (Figure 2A). Similar differences were observed at 15.5 and 18.5 dpc (Figure 2B and 2C).

### EG-VEGF Treatment Decreased Trophoblast Invasion Into the Maternal Decidua

Structural analysis showed that placentae from EG-VEGF–treated mice showed a significant decrease in the size of their decidua, suggesting a direct effect of EG-VEGF on trophoblast invasion. We further analyzed the invasive process in the placenta of mice treated at 12.5 dpc. In Figure 3A, trophoblast staining by cytokeratin was compared between saline-treated (a and b) and EG-VEGF–treated (c and d) placentae. Photographs in Figure 3C report periodic acid-Schiff staining in the saline-treated (e and f) and EG-VEGF–treated (g and h) placentae, respectively. Immunoreactivity for both cytokeratin 7 and periodic acid-Schiff stainings showed that the number of trophoblast cells that migrated into the decidua was significantly lower in the treated condition. A quantification of the number of invading cells is reported in Figure 3B and 3D.

In a previous study using human placental explant, we have shown that EG-VEGF controls extravillous trophoblast invasion via PROKR2 and not PROKR1.12 In this study, comparison of placental levels of PROKR1 and PROKR2 in saline- and EG-VEGF–treated animal showed that placenta from EG-VEGF–treated dams exhibited higher levels of PROKR2 and not PROKR1 (Figure S1 in the online-only Data Supplement).

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**Table. List of Primers Used for Real-Time Reverse Transcripase Polymerase Chain Reaction**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward (F)/Reverse (R)</th>
<th>T_m, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mash2</td>
<td>5'-GGTGAACCTGGTGAGGACCTA-3' / 5'-TCCGGAATGGAAGATGCACG-3'</td>
<td>56</td>
</tr>
<tr>
<td>Gcm1</td>
<td>5'-CGATGTTAAGACTGCCCTAGA-3' / 5'-GCTTCCCTGTGGAGGACATCGC-3'</td>
<td>57</td>
</tr>
<tr>
<td>Proliferin</td>
<td>5'-TGTTGGACAGAGGATGCTACG-3' / 5'-GTAGTTGGACAGGACCTGGTCG-3'</td>
<td>54</td>
</tr>
<tr>
<td>Protactin</td>
<td>5'-AAGTCCTACAGAGGTGACTGCACTA-3' / 5'-ACGCACTAGATCCTGCAAGG-3'</td>
<td>58</td>
</tr>
<tr>
<td>Hand1</td>
<td>5'-TCACAGCTACATCGCTACTTGA-3' / 5'-AGCCAGTGCTGGATTTAATCCT-3'</td>
<td>59</td>
</tr>
<tr>
<td>RPL13</td>
<td>5'-AGGGTGCTGGATTACAAGAAA-3' / 5'-AGTGACTCGGATTGGTCTGCC-3'</td>
<td>57</td>
</tr>
</tbody>
</table>

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![Figure 2.](http://hyper.ahajournals.org/)

**Figure 2.** Endocrine gland–derived vascular endothelial growth factor (EG-VEGF) treatment affects the layer surface of placental zones. **A-C.** Analysis of the placental zones of saline and EG-VEGF placentas collected at 12.5, 15.5, and 18.5 days post coitus (dpc), respectively. For each group, 2 placental sections per animal were analyzed. At 12.5 dpc, saline n=5 (a) and EG-VEGF n=3 (b). At 15.5 dpc, saline n=7 (c) and EG-VEGF n=7 (d). At 18.5 dpc, saline n=6 (e) and EG-VEGF n=8 (f). Surfaces of the 3 layers were measured on parasagittal sections for each placenta. Data represent mean±SEM (*P<0.05). ns indicates nonsignificant. 

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Evidence for Sustained Placental Hypoxia in EG-VEGF–Treated Placentae

To assess the degree of hypoxia in EG-VEGF–treated placentae, we compared the levels of CAIX expression in saline-treated and EG-VEGF–treated placentae. Figure 3E shows representative photographs of placental sections from saline-treated (a) and EG-VEGF–treated mice (b) at 15.5 dpc. CAIX staining was stronger in the EG-VEGF–treated placental sections compared with controls. The strongest staining was observed in the junctional zone. Comparison of the levels of CAIX protein expression by Western blot analysis substantiated the difference observed in situ (Figure 3F and 3G).

Changes in Placental Gene Expression

To determine whether the architectural abnormalities detected in 15.5-dpc placentae from EG-VEGF–exposed animals were linked to quantitative changes in gene expression, quantitative real-time reverse transcription polymerase chain reaction analyses were performed for several key trophoblastic genes with specific established roles in the formation of the placental structure. We evaluated the expression of Hand1, a protein that promotes differentiation of trophoblast giant cells; prolactin and prolactin, 2 markers for invasive trophoblasts; Mash2, a protein required for the maintenance of giant cell precursors and a marker for spongiotrophoblast; and Gcm1, an important protein for labyrinth branching. Exposure of gravid mice to EG-VEGF did not affect placental expression of Mash2. However, we observed significant decreases in proliferin, Hand1, prolactin, and Gcm1 (Figure 4A–4D). Taken together, these data suggest that EG-VEGF treatment affects trophoblast differentiation toward an invasive phenotype.

EG-VEGF Treatment Induces a Full Spectrum of PIH-Like Symptoms in the Gravid Mice at 15.5 and 18.5 dpc

On the basis of the aforementioned results, we predicted that EG-VEGF–treated gravid mice will develop pathogenesis of PIH. Key hallmarks of PIH include hypertension and proteinuria. First, we compared maternal arterial blood pressure in saline-treated and EG-VEGF–treated mice. Every other day, blood pressure was measured until 18.5 dpc. The graph in Figure 5A reports maternal arterial blood pressure at 8.5, 15.5, and 18.5 dpc. There was a significant increase in maternal arterial blood pressure at 15.5 and 18.5 dpc in the EG-VEGF–treated mice albeit much higher increase at 15.5 dpc than at 18.5 dpc. Second, we assessed urinary albumin excretion (normalized to creatinine). There was a significant increase in the albumin to creatinine ratio both at 15.5 and 18.5 dpc in the EG-VEGF–treated mice (Figure 5B). Another prominent clinical feature of PIH is the alteration in the expression and production of antiangiogenic factors by the placenta. We compared circulating sFlt1 and sEng in saline-treated and EG-VEGF–treated gravid mice at 12.5, 15.5, and 18.5 dpc. There was a significant increase of both antiangiogenic factors in EG-VEGF–treated animals (Figure 5C and 5D). The difference in sFlt1 levels was significantly different.
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as early as 12.5 dpc. Conversely, the increase in sEng was observed later on at 15.5 and 18.5 dpc. Comparison of sFlt1 protein expression between control and treated animals was also assessed at the placental level. A significant increase was observed when targeting the 130-kD form, corresponding the secreted soluble form of Flt (Figure S1).

A previous study has demonstrated that the proteinuria observed in PIH mice models with associated elevated sFlt1 and sEng was accompanied by marked renal histological changes. Kidney analysis of saline- and EG-VEGF–treated animals at 15.5 and 18.5 dpc revealed that the glomeruli of EG-VEGF–treated mice exhibited signs of focal and segmental sclerosis (Figure 6A). Quantification of scarring glomeruli in the 2 groups showed that EG-VEGF–treated mice exhibited a significant increase in abnormal glomeruli both at 15.5 and 18.5 dpc (Figure 6B). To better characterize the abnormal phenotype of the glomeruli, we compared the levels of expression of a marker of cell proliferation, proliferating cell nuclear antigen. Representative photographs on Figure 6C show that glomeruli from EG-VEGF–treated mice exhibited increased number of proliferating cell nuclear antigen–positive nuclei, suggesting increased cell proliferation in these structures. EG-VEGF effect on the kidney function was secondary to its effects on the placenta as similarly treated male mice had normal kidney structure and function (Figure S2).

Discussion

By mimicking an increase in circulating EG-VEGF beyond 11.5 dpc, similar to that observed beyond the first trimester of pregnancy in women with pathological pregnancies, we created a new mouse model of PIH. This statement is based on several key findings. First, EG-VEGF inhibited trophoblast invasion, a key process that ensures the establishment of the fetomaternal circulation. EG-VEGF function during early pregnancy is important as the embryo is not protected against potential reactive oxygen species present in the oxygenated maternal blood. However, maintenance of its production over the first trimester seems to be harmful for the establishment of the fetomaternal circulation. Second, placenta from EG-VEGF–treated mice exhibited increased hypoxia, a hallmark of stressed

Figure 4. Endocrine gland–derived vascular endothelial growth factor (EG-VEGF) treatment effect on the mRNA expression of proliferin, Hand1, prolactin, Gcm1, and Mash2 in the placenta. mRNA was isolated from whole placentas collected at 15.5 days post coitus (saline, n=7; EG-VEGF, n=7). Expression of the indicated genes was analyzed by quantitative real-time reverse transcription polymerase chain reaction, plotted in arbitrary units and normalized to RPL13. Data are presented as mean±SEM. (*P<0.05). ns indicates nonsignificant.
placentae. Although several reports have discussed the role of low-oxygen tension in PIH development, direct in vivo evidence linking increased placental hypoxia with PIH was still unsatisfactory. Third, placentae from EG-VEGF–treated dams exhibited decreased expression in key genes known to be involved in trophoblast differentiation toward an invasive phenotype. Gene analysis also showed a decrease in Gcm1, an important protein that controls labyrinth branching, suggesting that EG-VEGF effects on trophoblastic invasion affected fetoplacental growth. However, the present model did not exhibit intrauterine growth restriction, as often observed in preeclampsia. This might well be explained by the fact that EG-VEGF is also a survival factor for trophoblast cells. Although EG-VEGF maintenance over the first trimester is a causative of placental stress, its elevation during the third trimester is proposed as compensatory mechanism to ensure a successful progress in pregnancy. In the present model, a significant increase in the placental efficiency in the treated group was observed at 18.5 dpc (Figure S3), suggesting in situ placental adaptation, which was substantiated by an increase in placental vascularization and proliferation in the labyrinth layer (Figure S4). Fourth, EG-VEGF induced an increase in local and circulating sFlt1, as well as circulating sEng. 2 antiangiogenic factors with established effects on the maternal vascular system and kidney function and known to be increased in preeclampsia.

Preeclampsia is commonly described as a disease of 2 stages. Stage 1 occurs during trophoblast invasion, and stage 2 consists of the clinical manifestations. The great majority of animal models aim to mimic the second stage. We believe that our model mimics more stage 1 than stage 2 of the disease. Although the present model does not recapitulate full symptoms of a typical preeclampsia observed in women and to certain extent in strong models, such as the RUPP, sFLT1, and sEng animal models, it does bring strong information about the mechanism of stage 1 development of PIH.

Interestingly, EG-VEGF–treated male mice did not show any sign of hypertension or kidney dysfunction, suggesting that EG-VEGF effects on the kidney function are secondary to its effects on the placenta. Also, we observed that the gravid-treated mice developed glomerular focal and segmental sclerosis rather than the classical glomeruli endotheliosis observed in preeclampsia. Importantly, the kidneys of EG-VEGF–treated

Figure 5. A, Endocrine gland–derived vascular endothelial growth factor (EG-VEGF)–treated mice develop gestational hypertension. Summary of maternal arterial blood pressure (MAP) recorded longitudinally before and during EG-VEGF treatment (arrow). Data are expressed as mean±SEM (*P<0.05). B, EG-VEGF–treated mice exhibit proteinuria during gestation. Summary of 24-hour urinary albumin/creatinine ratio are reported for days 8.5, 15.5, and 18.5 of gestation. Data are expressed as mean±SEM (*P<0.05). C, EG-VEGF treatment increased circulating soluble fms-like tyrosine kinase 1 (sFlt1; *P<0.05). D, EG-VEGF treatment increased circulating soluble endoglin (sEng). Data are expressed as mean±SEM (*P<0.05). dpc indicates days post coitus; and ns, nonsignificant.

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mice displayed higher proliferation rate, suggesting a long-term beneficial effect of EG-VEGF on kidney function. These results are in agreement with the observations that the knock-down of PROKR1 resulted in abnormal kidney functions. Unpublished data from our group using a renal distal tubule cell line (KAC3) demonstrated that EG-VEGF increased their proliferation and survival. Hence, EG-VEGF maintenance may have an alternate but direct beneficial effects on the kidney compared with the deleterious sFlt1 effects. Altogether our findings have demonstrated that EG-VEGF induces a PIH phenotype if maintained beyond the first trimester of pregnancy and provides evidence to suggest that maintenance of elevated EG-VEGF during the second and third trimesters of pregnancy contributes to the attenuation of this pathogenesis. These in vivo observations are consistent with our previous studies using human tissues during normal pregnancy and also in pathological pregnancies. Therefore, this study provides novel insights into the fine role of EG-VEGF in the development of PIH and the attenuation of its pathological symptoms, once the pathology is established.

Perspectives

The present study provokes 2 perspectives to be verified in regard to the proposed cause or consequent role of EG-VEGF in relation to the pathogenesis of PIH. The direct causative role can be verified by a dual treatment of the gravid mice at 11.5 dpc by EG-VEGF and its receptor antagonists. Importantly, these antagonists have been shown to reduce EG-VEGF actions and to exhibit analgesic properties. The present study will also be a support of an ongoing clinical study that aims at demonstrating an association between increased levels of EG-VEGF by the end of the first trimester and the occurrence of pregnancy pathologies. The consequent role of increased EG-VEGF on the attenuation of PIH symptoms could be tested using a mouse model of established preeclampsia animal. This model could be the RUPP, sFlt1, or STOX1 model.

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Disclosures

None.

References

Novelty and Significance

What Is New?

- A new model of pregnancy-induced hypertension is now available to test new therapeutic agents.
- An excess of endocrine gland-derived vascular endothelial growth factor (EG-VEGF) over the first trimester can cause pregnancy-induced hypertension.
- EG-VEGF effects on the kidney function during pregnancy are secondary to its effects on the placenta.

What Is Relevant?

- We have demonstrated the molecular mechanisms by which EG-VEGF contributes to the development of pregnancy-induced hypertension.

- EG-VEGF effects on the kidney function are secondary to its action on the placenta.

Summary

This study constitutes a strength of an ongoing clinical study that aims at demonstrating an association between increased levels of EG-VEGF by the end of the first trimester and the occurrence of pregnancy pathology.
Sustained Endocrine Gland–Derived Vascular Endothelial Growth Factor Levels Beyond the First Trimester of Pregnancy Display Phenotypic and Functional Changes Associated With the Pathogenesis of Pregnancy-Induced Hypertension

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Sustained EG-VEGF levels beyond the first-trimester of pregnancy display phenotypic and functional changes associated with the pathogenesis of pregnancy-induced hypertension.

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**Short title:** Increased EG-VEGF levels cause PIH

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*equal contribution

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Supplemental data

Results

**EG-VEGF effects on kidney structure and function are secondary to its adverse effects on placental function.**

EG-VEGF-treated mice exhibited both placental and kidney dysfunctions. However, whether EG-VEGF effects on the kidney are secondary to its effects on the placenta or are due to direct effects of this factor on the kidney function remained to be determined. To address this question, we treated male mice under the same conditions as those used with gravid mice. Arterial pressure, kidney function and histology were assessed. Fig S2A-C shows that neither arterial blood pressure nor renal function or histology were affected by EG-VEGF treatment suggesting that EG-VEGF effects on kidney are secondary to their effects on placental function.

**Placental adaptation to rescue pregnancy outcome.**

Because PE is often associated with IUGR, we compared placental and fetal weight as well as placental efficiency at 15.5 and 18.5 dpc in saline- and EG-VEGF-treated animals. Fig. S3 reports comparisons between the litter sizes, placental weights, fetal weights and placental efficiencies under both conditions. There was no change in the litter size between control and treated groups at both gestational ages examined. At 15.5 dpc, no change could be observed in all parameters examined. However at 18.5dpc, we observed a significant decrease in placental weight with no change in the fetal weight. A significant increase in the placental efficiency in the treated group could be observed at this gestational age, suggesting placental adaptation in response to insults caused by the abnormal maintenance of circulating EG-VEGF. Placental adaptation at 18.5 dpc was substantiated *in situ* by the observation of an increase in placental vascularization and proliferation in the labyrinth layer, Fig S4.
Figure S1: EG-VEGF treatment increased placental PROKR2 and sFLT1 proteins expression. 5A, 5B and 5C show comparisons and quantifications of PROKR1, PROKR2 and sFLT1 protein levels in saline and in EG-VEGF placental extracts, respectively. Standardization was performed by β-actin. Data represent the mean ±SEM. (* p < 0.05).
Figure S2. EG-VEGF treated male mice did not develop preeclampsia symptoms. A reports EG-VEGF serum levels in male mice treated or not with EG-VEGF. A total of 14 serum samples were analyzed (CTL, n=7) and (+EG-VEGF, n=7). EG-VEGF contents were measured by ELISA. Data represent the mean ±SEM. (* p < 0.05). B shows a summary of MAP that was recorded longitudinally before, and during EG-VEGF treatment (arrow). C compares a summary of 24-hour urinary Albumin/creatinine ratio. Mice were placed in metabolic cages, and 24-hour urine samples were collected over 2 days. Data are expressed as mean ± SEM. D shows representative photomicrographs of hematoxylin-eosin-stained kidney sections from a saline and EG-VEGF treated male mice.
Figure S3. EG-VEGF treated mice exhibit placental deregulations at 18.5 dpc. (A,E, I) compare control and EG-VEGF treated gravid mice for their litter sizes at 12.5, 15.5 and 18.5 dpc, respectively. (B,F, J) compare control and EG-VEGF treated gravid mice for their fetal weights at 12.5, 15.5 and 18.5 dpc, respectively. (C,G, K) compare control and EG-VEGF treated gravid mice for their placental weights at 12.5, 15.5 and 18.5 dpc, respectively. (D, H, L) compare control and EG-VEGF treated gravid mice for their placental efficiencies at 12.5, 15.5 and 18.5 dpc, respectively. For each gestational age at least 12 control and 8 EG-VEGF treated mice were included. Data are expressed as mean ± SEM. (*p < 0.05).
Figure S4: EG-VEGF increased placental angiogenesis and trophoblast proliferation in the labyrinth layer of 18.5 dpc treated mice. A shows representative photomicrographs of placental sections from a saline and EG-VEGF treated 18.5 dpc mice stained with anti-CD31. B compares CD31 protein expression levels in saline (n=) and EG-VEGF treated mice (n=). C shows representative photomicrographs of placental sections from a saline and EG-VEGF treated 18.5 dpc mice stained with anti-PCNA. D compares PCNA protein expression levels in saline (n=) and EG-VEGF treated mice (n=). Data are expressed as mean ± SEM. (*p < 0.05).