Glyceryl Trinitrate Inhibits Hypoxia-Induced Release of Soluble Fms-Like Tyrosine Kinase-1 and Endoglin from Placental Tissues

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Preeclampsia is associated with increased circulating levels of proinflammatory molecules, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng). On release by an inadequately perfused placenta into the maternal circulation, these molecules cause systemic endothelial dysfunction and the associated hypertension and proteinuria that characterize preeclampsia. We previously showed that glyceryl trinitrate (GTN) inhibits hypoxia/reoxygenation-induced apoptosis in the syncytiotrophoblast of term chorionic villi explants. Herein, we demonstrate that GTN inhibits the release of sFlt-1 and sEng from term chorionic villi explants exposed to hypoxia. Although transcript levels and secretion of sFlt-1 and sEng increased in explants exposed to hypoxia, low concentrations of GTN significantly inhibited the hypoxia-induced expression of these molecules at the mRNA and protein levels. Treatment of explants with GTN also prevented the hypoxia-induced accumulation of hypoxia-inducible factor-1α, a key mediator of cellular adaptations to hypoxia. Furthermore, knockdown of hypoxia-inducible factor-1α inhibited the hypoxia-induced secretion of sFlt-1 and sEng. This study provides evidence that hypoxia induces the release of sFlt-1 and sEng in the placenta via a mechanism that is inhibited by low concentrations of GTN. Our findings indicate that GTN may have potential applications in the treatment and/or prevention of preeclampsia. (Am J Pathol 2011, 178:2888–2896; DOI: 10.1016/j.ajpath.2011.02.013)
Similar to signaling by VEGF and placental growth factor, TGF-β signaling plays an important role in the maintenance of vascular function and homeostasis. In preeclampsia, high levels of sEng in the maternal circulation correlate with increased expression of Eng in the placenta. Although the precise role of NO types in cancer cells, such as invasiveness, metastasis, and drug resistance. 

Placental hypoxia may play a major role in the pathological characteristics of preeclampsia. Hypoxia increases the secretion of sFlt-1 and sEng from first- and second-trimester chorionic villi explants. Although hypoxia also increases the secretion of sFlt-1 by third-trimester explants, its effect on sEng secretion by third-trimester explants has not been determined. Because the clinical signs of preeclampsia only appear in the second half of pregnancy, it is possible that a hypoxia-induced increase in sFlt-1 and sEng secretion in the second and third trimesters of gestation contributes to the pathological characteristics of preeclampsia.

The hypoxia response pathway represents a potential therapeutic target for inhibiting placental secretion of sFlt-1 and sEng. A key player that mediates many adaptive responses to hypoxia is the transcriptional activator hypoxia-inducible factor (HIF)-1. This transcription factor is a basic helix-loop-helix-PAS domain protein composed of α and β subunits. Although the HIF-1β subunit is constitutively expressed, the levels of HIF-1α increase in response to hypoxia. The stability of HIF-1α is regulated by hydroxylation of proline residues 402, 564, or both in an oxygen-dependent reaction. Proline hydroxylation of HIF-1α promotes the binding of the von Hippel–Lindau protein, leading to ubiquitination and proteasomal degradation of HIF-1α.

There is evidence that relatively low concentrations of nitric oxide (NO) inhibit the accumulation of HIF-1α under hypoxia, thereby interfering with HIF-1–mediated adaptive responses. Low concentrations (lower than micromolar) of NO mimetics [e.g., glyceryl trinitrate (GTN)] inhibit the hypoxia-induced acquisition of malignant phenotypes in cancer cells, such as invasiveness, metastasis, and drug resistance. Although the precise role of endogenous NO in normal and pathological pregnancy is unclear, there is evidence that decreased endogenous NO availability contributes to the pathophysiological characteristics of preeclampsia. A previous study demonstrated that low concentrations of GTN inhibit apoptosis of the syncytiotrophoblast in chorionic villi explants exposed to hypoxia/reoxygenation (H/R). In the present study, we determined the effect of administration of low concentrations of GTN on the secretion of sFlt-1 and sEng from term chorionic villi explants exposed to hypoxia.

**Materials and Methods**

**Collection and Culture of Chorionic Villi Explants**

Human term placentas (n = 22) were obtained from uncomplicated pregnancies immediately after caesarean deliveries at Kingston General Hospital, Kingston, ON. Placentas were collected with the approval of the Queen’s University Research Ethics Board. Explants of chorionic villi were prepared as previously described and incubated in 1.3 mL of serum-free RPMI 1640 medium (Invitrogen, Burlington, ON). For exposures to hypoxia, explants (approximately 60 per experiment) treated with or without GTN (10 nmol/L or 1 μmol/L; Sabex, Boucherville, QC) were incubated at 37°C for 24 hours in a humidified Plexiglas chamber flushed with a gas mixture of 5% CO₂ and 95% N₂. Oxygen concentrations within the chamber were maintained at 0.5% (PO₂ = 3.8 mm Hg) by oxygen regulators (ProOx 110; Biospherix Inc., Lacona, NY). Controls consisted of explants incubated in either 8% O₂ (PO₂ = 60 mm Hg, physiological concentrations) or 20% O₂ (PO₂ = 152 mm Hg) and 5% CO₂ for 24 hours. Explants were then collected or exposed to conditions of reoxygination (20% O₂) for 3 additional hours. At the end of all incubations, placental tissues were either flash frozen for molecular analysis or fixed in 4% paraformaldehyde for histological analysis. Media of cultured explants were collected, centrifuged for 10 minutes at 8000 x g, divided into small tubes, and stored at -80°C until further analysis.

**Immunohistochemistry**

Randomly selected paraffin-embedded sections of chorionic villi were deparaffinized and subjected to antigen retrieval by heating them in a microwave oven for 22 minutes in citrate buffer (0.1 mol/L sodium citrate, pH 6.0). Primary antibodies used were goat polyclonal anti-human Eng or Flt-1 antibody (1 μg/mL; R&D Systems, Minneapolis, MN), mixed with mouse anti-human pancytokerin antibody (1:100; Sigma-Aldrich, St Louis, MO) and incubated at 4°C overnight. Secondary antibodies were a mix of donkey anti-mouse (Alexa Fluor 568) and donkey anti-goat (Alexa Fluor 488) antibodies (1:500 each; Invitrogen, Carlsbad, CA). Slides were mounted with medium (VECTASHIELD Mounting Medium) containing DAPI (Vector Laboratories, Inc., Burlingame, CA). Micrographs were taken using a microscope (Zeiss Imager A1 Microscope; Carl Zeiss Canada Ltd, Toronto, ON) and software (Axiovision v 4.7.1.0; Carl Zeiss Canada Ltd).

**Western Blot Analysis of HIF-1α**

To assess the ability of chorionic villi to respond to hypoxia, the levels of HIF-1α were determined by Western blot analysis of flash-frozen chorionic villi preexposed to 0.5%, 8%, or 20% O₂ for 24 hours (n = 3 to 6 placentas). Primary antibodies used were mouse monoclonal antibody against HIF-1α (1:250; BD Biosciences, San Diego, CA), goat polyclonal anti–TGF-β1 (1:1000, R&D Systems), and monoclonal anti–β-actin antibody (clone AC-15, 1:8000; Bio-Rad Laboratories Ltd, Mississauga, ON, Canada). Goat anti-mouse antibody (1:5000, Vector Laboratories Inc.) labeled with horseradish peroxidase was used as a secondary antibody. The relative intensities of bands were determined by densitometry using software (AlphaErase; α Innotech Corp, San Leandro, CA).
Knockdown of HIF-1α in Chorionic Villi Explants

To down-regulate HIF-1α expression in chorionic villi, HIF-1α small-interfering RNA (siRNA) was introduced into explants using a transfection reagent (siPORT NeoFX; Ambion Inc., Austin, TX). This HIF-1α siRNA was validated and targets exon 5 of the human HIF-1α gene (number 42840; Ambion Inc.). Explants were transfected with 200 nmol/L HIF-1α siRNA or negative control siRNA 2 (Silencer; Ambion Inc.) and then incubated in a standard incubator at 5% CO₂ and 20% O₂ (151 mm Hg) for 24 hours. After this incubation, the culture medium was replaced with fresh RPMI 1640 medium (Invitrogen, Burlington, ON, Canada) and explants were further incubated for 24 hours in either 20% or 0.5% O₂, as previously described.

**Determination of sFlt-1 and sEng Levels in the Culture Media**

To determine the levels of sFlt-1 or sEng in the culture media, kits (Duoset ELISA Development System kits; R&D Systems) were used according to the manufacturer’s instructions.

**Real-Time PCR**

Total RNA was isolated using a kit (High Pure RNA Isolation Kit; Qiagen, Valencia, CA), according to the manufacturer’s protocol. Total RNA, 1 μg, was reverse transcribed with a kit (OmniScript RT Kit; Qiagen) using a random hexamer (Cortex, Kingston). Real-time PCR was performed with a system (LightCycler 480 Real-Time PCR System; Roche, Mississauga, ON) using a mix (SYBR Green PCR Master Mix; Roche) and primers, as follows: β-actin, 5′-TGGGACGACATGGAGAATA-3′ (sense) and 5′-GAGGCGTACAGGGATAGCAC-3′ (antisense); Flt-1, 5′-GCACCCTTGTGTTGCTGAC-3′ (sense) and 5′-TG-GAATTCGTGCTGTTCTCGTCC-3′ (antisense); sFlt-1, 5′-GCACCTGCAACAAAAAGGC-3′ (sense) and 5′-CCAG-GAATGTATACACAGG-3′ (antisense); Eng, 5′-GCTGGATGAGCCGGGAGCTCCTGCTG-3′ (sense) and 5′-CA-CAGGCTGAAAGTCACAATGGACTG-3′ (antisense); and HIF-1α, 5′-GCTGGATGAGCCGGGAGCTCCTGCTG-3′ (sense) and 5′-GGTCAAGTATGATCAAGTCCA-3′ (antisense). Real-time reaction conditions for all sets of primers were as follows: 94°C for 5 minutes, followed by 60 cycles at 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 30 seconds. The intensity of dye (FastStart SYBR Green) was analyzed using software (LightCycler 480; Roche). Transcript levels were normalized against β-actin mRNA levels.

**Statistical Analysis**

For statistical analysis of enzyme-linked immunosorbent assay and quantitative PCR results, one-way analysis of variance was performed and significant differences between groups were determined using Tukey’s multiple comparisons post hoc test. All statistical tests were two sided, and differences were considered significant at P < 0.05.

Results

**Immunolocalization of Flt-1 and Eng in Chorionic Villi Explants**

To determine the potential source of secreted Flt-1 and Eng, immunofluorescence using polyclonal antibodies against Flt-1 and Eng was performed on sections of chorionic villi explants incubated in 20% O₂ (see Supplemental Figure S1, A and E, at http://ajp.amjpathol.org). Cytokeratin was used as a marker for trophoblast (see Supplemental Figure S1, B and F, at http://ajp.amjpathol.org). Both Flt-1 and Eng colocalized with cytokeratin primarily to the syncytiotrophoblast layer (see Supplemental Figure S1, D and H, at http://ajp.amjpathol.org), indicating that this tissue is a potential source of the secreted molecules. We also observed that Flt-1, but not Eng, is expressed in the fetal vessels within the villous stroma, indicating that fetal vessels are another potential source of sFlt-1 but not of sEng (see Supplemental Figure S1, D versus H, respectively, at http://ajp.amjpathol.org).

**Effect of Hypoxia on the Levels of sFlt-1 and Eng mRNA and Secreted Protein**

Previous studies14,15,17,28 reported hypoxia-induced sFlt-1 and sEng secretion by trophoblast cell lines and first-trimester chorionic villi explants. Given that preeclampsia is mostly a disease of the second half of pregnancy, we examined the effect of hypoxia on the mRNA levels of Flt-1 variants and Eng by term chorionic villi explants. The secreted form of Flt-1 either can be encoded by a spliced variant of the Flt-1 (spliced Flt-1) gene or, less frequently, is the product of the cleaved ectodomain of the Flt-1 receptor (receptor Flt-1).29,30 To determine whether a specific variant of Flt-1 is responsive to hypoxia in term villous explants, we examined both species of Flt-1 mRNA using real-time PCR. Exposure to 0.5% O₂ increased the expression of both the spliced Flt-1 and receptor Flt-1 mRNA species compared with exposure to either 20% or 8% O₂ (Figure 1A). However, although the expression of the spliced Flt-1 variant increased several thousandfold, the levels of transcript encoding the receptor Flt-1 variant increased by only approximately twofold. Consequently, the ratio of spliced Flt-1/receptor Flt-1 significantly increased in chorionic villi exposed to hypoxia compared with chorionic villi exposed to either 20% or 8% O₂ (Figure 1A; P < 0.001 for both). This ratio is an indicator of increased bioavailability of the spliced (secreted) Flt-1 variant compared with nonspliced Flt-1 receptor in chorionic villi exposed to hypoxia. Exposure of chorionic villi to hypoxia (0.5% O₂) resulted in a fivefold and a threefold increase in Eng mRNA levels compared with exposure to 20% and 8% O₂, respectively (Figure 1B; P < 0.001 for both). Compared with exposure to 20% O₂, exposure to 8% O₂ did not result in statistically significant increases in the levels of sFlt-1 and sEng mRNA. To further characterize the effect of hypoxia on the secretion of sFlt-1 and sEng, we performed an enzyme-
linked immunosorbent assay on the media of explant cultures. Explants exposed to 0.5% O₂ for 24 hours secreted significantly higher levels of sFlt-1 and sEng than explants incubated in 20% or 8% O₂ (Figure 2, A and B). Although the data shown in Figure 2 are expressed as pictograms of sFlt-1 or sEng per microgram of protein in the media, differences in the reported concentrations of these molecules were not the result of differences in the total amount of protein secreted by chorionic villi; the latter was similar across the different treatment groups (see Supplemental Figure S2 at http://ajp.amjpathol.org).

Compared with exposure to 20% O₂ alone, exposure of chorionic villi to H/R (24 hours in 0.5% O₂, followed by 3 hours in 20% O₂) did not significantly increase the detectable levels of sFlt-1 and sEng in the culture medium (see Supplemental Figure S3, A and B, at http://ajp.amjpathol.org). Accordingly, compared with exposure to hypoxia for 24 hours, subsequent reoxygenation of chorionic villi explants (3 hours in 20% O₂) resulted in a significant decrease in the levels of detectable sFlt-1 and sEng (see Supplemental Figure S4, A and B, at http://ajp.amjpathol.org; P < 0.05 for both).

**Effect of GTN on the Hypoxia-Induced Increases in sFlt-1 and sEng mRNA Levels and Secretion**

The hypoxia-mediated increases in sFlt-1 and sEng mRNA accumulation and protein secretion were significantly inhibited by GTN at concentrations of 10 nmol/L or 1 μmol/L (Figure 1, A and B, and Figure 2, A and B). The inhibitory effect of GTN at 1 μmol/L was confirmed by immunofluorescence microscopy of explants using polyclonal antibodies against Flt-1 or Eng. Compared with exposure to hypoxia alone, Flt-1 and Eng immunofluorescence intensity under hypoxic conditions was substantially decreased after treatment with 1 μmol/L GTN (Figure 2, C and D).

**Effect of Hypoxia and GTN on HIF-1α Accumulation**

To elucidate the mechanism by which GTN inhibits hypoxia-induced secretion of sFlt-1 and sEng, we examined the effect of GTN on the accumulation of HIF-1α in explants incubated in 0.5% O₂. HIF-1α levels were significantly increased in explants exposed to 0.5% O₂ compared with HIF-1α levels in explants exposed to 20% O₂ (Figure 3, A and B: P < 0.01) or 8% O₂ (Figure 3, A and B: P < 0.05). Treatment with GTN at a concentration of 1 μmol/L or 10 nmol/L significantly inhibited the accumulation of HIF-1α in explants exposed to 0.5% O₂ (Figure 3, A and B: P < 0.001 and P < 0.01, respectively). Consistent with the inhibitory effects of GTN on sFlt-1 and sEng secretion, the most pronounced suppression of HIF-1α accumulation was achieved with GTN at a concentration of 1 μmol/L.

**Effect of HIF-1α Knockdown on the Secretion of sFlt-1 and sEng**

To determine whether HIF-1 mediates the hypoxia-induced secretion of sFlt-1 and sEng, we knocked down HIF-1α mRNA in chorionic villi explants. Villi exposed to 0.5% O₂ significantly expressed higher levels of HIF-1α mRNA compared with villi exposed to 20% O₂ or villi exposed to 0.5% O₂ and transfected with HIF-1α siRNA (Figure 4A: P < 0.001 for both). Chorionic villi with higher HIF-1α expression levels (ie, exposed to 0.5% O₂) secreted more sFlt-1 and sEng than those with lower HIF-1α mRNA levels (ie, exposed to 20% O₂; Figure 4, B and C: P < 0.05 and P < 0.01, respectively). Knockdown of HIF-1α with siRNA inhibited the hypoxia-induced secretion of sFlt-1 and sEng (Figure 4, B and C: P < 0.001 for...
Figure 2. Effect of GTN on the secretion and expression of sFlt-1 and sEng by chorionic villi explants exposed to hypoxia. A and B: Relative sFlt-1 and sEng levels, respectively, in the medium of explant cultures (six explants per placenta, with a minimum of three placentas per treatment). Concentrations (in ng/mL) were divided by the total amount of protein (in µg/mL) in the media. Error bars represent SEM. The lines in A and B identify the comparison groups by the vertical lines pointing to the corresponding bars. C and D: Localization of Flt-1, Eng, cytokeratin (trophoblast marker), and nuclei (DAPI) by fluorescence microscopy. Far right: The merged images with all three markers combined, with yellow representing areas of colocalization of sFlt-1 or Eng with cytokeratin, are shown. Top: Explants exposed to hypoxia (0.5% O₂). Bottom: Explants exposed to hypoxia in the presence of 1 µmol/L GTN. *P < 0.05, **P < 0.01, and ***P < 0.001. Transverse lines link two groups to indicate statistical significance. Scale bars = 10 µm. ELISA indicates enzyme-linked immunosorbent assay.
Furthermore, explants transfected with HIF-1α siRNA secreted significantly lower levels of sFlt-1 and sEng than explants transfected with scrambled negative control siRNA (Figure 4, B and C: *P < 0.05 and **P < 0.01, respectively). These results indicate that HIF-1α is a key mediator of the hypoxia-induced secretion of sFlt-1 and sEng. These findings also indicate a hypoxia-induced up-regulation of HIF-1α at the mRNA level, which was previously reported.31 However, our findings do not exclude the possibility that hypoxia may also increase HIF-1α levels by increasing protein stability.23,32

Discussion
The main novel finding that we report herein is that hypoxia-induced secretion of sFlt-1 and sEng by chorionic villi explants can be inhibited by low concentrations of GTN. Given that hypoxia and increased circulating levels of sFlt-1 and sEng contribute to the pathophysiological characteristics of preeclampsia, the results of this study indicate that administration of low doses of NO mimetics to women with preeclampsia or at a high risk of developing this complication may be a useful therapeutic or preventative strategy.

Other studies14,17,28 have focused primarily on determining the effect of hypoxia on placental sFlt-1 secretion; increased sFlt-1 secretion during preeclampsia may be part of a placental response to hypoxia, whereby sFlt-1 increases maternal blood pressure to improve placental perfusion. Hypoxia-mediated stimulation of sFlt-1 and sEng secretion by first-trimester chorionic villi explants was previously reported.15,17,28 However, the role of hypoxia in the regulation of sEng secretion is controversial. Although some studies28,33 have described a lack of effect of hypoxia on sEng secretion, others15,34 have found a stimulatory effect in first-trimester explants and preeclamptic term placentas but not in normal-term placentas. In light of our results, it is possible that sEng secretion is dependent on gestational age and length of exposure to hypoxia.
In women who later develop preeclampsia, increased circulating sFlt-1 and sEng levels are first detected in the late second and early third trimesters, respectively, before the clinical onset of disease. Thus, cultures of third-trimester explants may represent a more accurate model to study the effects of hypoxia on placental sFlt-1 and sEng secretion.

In our study, only hypoxia, and not H/R, resulted in increased detection of sFlt-1 and sEng in the culture medium. Although the effect of H/R on the secretion of sFlt-1 and sEng by chorionic villi was not previously examined, Cindrova-Davies reported a significant increase in tissue-associated sFlt-1 levels in chorionic villi after exposure to H/R. In our study, compared with exposure to hypoxia alone, exposure to conditions of reoxygenation resulted in decreased detectable levels of sFlt-1 and sEng. We cannot exclude that H/R increased the secretion of sFlt-1 and sEng by chorionic villi explants. It is possible that the decreased detection of these molecules after H/R was the result of sequestration by high levels of secreted VEGF and TGF-β, which can potentially interfere with the detection assay. In support of this concept, Western blot analysis revealed substantial increases in the levels of TGF-β1 in the culture medium of explants exposed to H/R compared with media from explants incubated in 20% or 0.5% O2 alone (see Supplementary Figure S4C at http://ajp.ampathol.org).

Although exposure of chorionic villi to hypoxia alone can lead to apoptosis, thereby resulting in the release of vasoactive molecules, we showed herein that the hypoxia-induced release of sFlt-1 and sEng can also be transcriptionally regulated. This conclusion is based on the observation that the hypoxia-induced expression and release of these molecules was mediated by HIF-1α. In our study, both HIF-1α protein and mRNA levels increased in chorionic villi exposed to hypoxia. Although HIF-1α levels are mostly regulated at the level of protein stabilization, there is evidence that hypoxia can increase the levels of HIF-1α mRNA in chorionic villi exposed to hypoxia.

Our results also revealed that the increased ratio of spliced Flt-1/receptor Flt-1 mRNA and the increase in Eng mRNA levels were much higher than the secreted protein levels. To our knowledge, this is the first report on the ratio of spliced Flt-1/receptor Flt-1 mRNA in the placenta and may, in part, explain the observed large increase in sFlt-1 mRNA levels. Differences between secreted protein and mRNA levels may be explained at the level of RNA stability and efficiency of the translational machinery.

Our findings support the results of previous studies showing that the syncytiotrophoblast is a major source of Flt-1 and Eng. We have demonstrated that the expression and secretion of Flt-1 and Eng from syncytiotrophoblast exposed to hypoxia can be inhibited by GTN. The rationale for testing the effect of GTN on the expression and release of these molecules was based on a previous study in which H/R-mediated apoptosis of term chorionic villi was significantly attenuated by low concentrations of GTN. It is possible that the effects of GTN, as an NO mimic, reflect the well-known actions of NO. At lower concentrations, such as those used in our study, NO activates soluble guanylyl cyclase to generate cGMP. Under certain conditions involving low oxygenation, NO can also inhibit mitochondrial respiration so that oxygen becomes available for the activation of prolyl hydroxylases and the degradation of HIF-1α. Herein, we showed that GTN was able to inhibit HIF-1α protein accumulation. The precise mechanism by which GTN inhibited HIF-1α accumulation requires further investigation. However, based on this observation, together with the finding that siRNA-mediated knockdown of HIF-1α prevented the hypoxia-induced release of sFlt-1 and sEng, we conclude that the inhibitory effect of GTN on the hypoxia-induced secretion of sFlt-1 and sEng was likely the result of inhibition of HIF-1 activity.

The pathophysiological characteristics of preeclampsia and other complications of pregnancy have been linked to a maternal immune imbalance with greater secretion of proinflammatory cytokines and a relative decrease in the levels of anti-inflammatory molecules, such as IL-10. Although the administration of IL-10 to chorionic villi explants did not affect the secretion of sFlt-1, treatment of explants with exogenous sFlt-1 increased IL-10 and tumor necrosis factor-α secretion. It is possible that, by increasing the secretion of tumor necrosis factor-α in the placenta, sFlt-1 contributes to the maternal inflammatory state characteristic of severe preeclampsia.

Women who have had preeclampsia are at increased risk of recurrence in a subsequent pregnancy. Despite the fact that the cause of preeclampsia is largely unknown, the increase in sFlt-1 and sEng levels in the maternal circulation is tightly linked to the pathophysiological characteristics of preeclampsia. Current therapeutic strategies have relied primarily on managing symptoms, with little evidence that any intervention alters the underlying pathophysiological characteristics. For example, antihypertensive and antiplatelet drugs have been used to treat severe hypertension and coagulopathies associated with preeclampsia. However, it is unclear that such approaches have an impact on other maternal and fetal comorbidities. Furthermore, a recent study revealed that antihypertensive drugs had no effect on placental secretion of sFlt-1 and sEng. There are few published studies on the use of GTN for the treatment of preeclampsia. Some studies have reported beneficial effects of GTN in preeclamptic patients with deficient uteroplacental blood flow. Other studies did not reveal the beneficial effects of this drug. In those studies, it is possible that patients developed tolerance to GTN because of continuous use of relatively high doses of this drug. It is also possible that only a subset of patients with preeclampsia benefit from GTN therapy (specifically, patients with hypoxic placetas and elevated serum levels of sFlt-1 and/or sEng).

In summary, our current study reveals a novel strategy to inhibit the secretion of sFlt-1 and sEng from placental tissues exposed to hypoxia and, therefore, has the potential of leading to novel modalities for the management and prevention of preeclampsia.
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