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Gasotransmitters

A Solution for the Therapeutic Dilemma in Preeclampsia?

Kim M. Holwerda, Marijke M. Faas, Harry van Goor, A. Titia Lely

Preeclampsia complicates 2% to 8% of all pregnancies and is a major contributor to maternal mortality worldwide. The only therapy is delivery, often before term.¹ Nitric oxide, carbon monoxide, and hydrogen sulfide are gasotransmitters that regulate vascular development, vascular tone, and affect antioxidant status.² Abnormalities in gasotransmitter signaling and production are linked to hypertension, atherosclerosis, and inflammation.² Drugs that enhance gasotransmitter signaling have proven therapeutic potential in the clinical and experimental setting.

Gasotransmitters are involved in the vascular adaptations of normal pregnancy,³⁻⁷ and experimental studies have shown that abnormal production is associated with preeclampsia.⁸⁻¹² The aim of this review is to give an overview of the role of gasotransmitters in the physiology of pregnancy and relate their aberrant production to preeclampsia. Specific emphasis will be put on and overview their therapeutic potential for preeclampsia.

Preeclampsia

Preeclampsia is defined by hypertension and proteinuria during the second half of gestation. Although the exact cause of preeclampsia is unknown, the placenta and the maternal inflammatory response play a key role in its pathogenesis.^{1,13} Preeclampsia is often described as a 2-stage disease. During placentation in normal pregnancy, trophoblasts invade into the endometrium and spiral arteries resulting in spiral artery dilation.¹ In preeclampsia, trophoblast invasion is incomplete resulting in inadequate spiral artery remodeling and placental hypoperfusion; this is stage 1 of the disease.¹ Subsequently, the oxygen-deprived placenta produces factors that enter the maternal circulation, causing maternal endothelial dysfunction and maternal immune response activation, leading to the signs of preeclampsia; this is stage 2 of the disease.¹

Some of the factors produced by the placenta are antiangiogenic factors, such as soluble fms-like tyrosine kinase receptor 1 (sFlt1).¹⁴ They are (partly) responsible for the maternal syndrome in preeclampsia because increased sFlt1 is associated with reduced levels of its circulating proangiogenic ligands, placental growth factor, and vascular endothelial growth factor (VEGF).¹⁴ In addition, preeclamptic women have less transforming growth factor- β caused by increased

placental production of soluble transforming growth factor- β coreceptor endoglin (sEng).¹⁵ Alterations in (anti)-angiogenic factors are thought to lead to endothelial dysfunction and consequently hypertension and proteinuria.^{14,15}

The systemic inflammatory response is activated during normal pregnancy and exaggerated in preeclampsia.¹³ An improper activated inflammatory response is linked to abnormal trophoblast invasion, endothelial cell damage, and renal dysfunction.¹³ Several proinflammatory factors, such as tumor necrosis factor- α and interleukin-6, are released by the preeclamptic placenta.¹⁶ In rat models, triggering of the inflammatory system, for example, by LPS (lipopolysaccharide) and tumor necrosis factor- α , leads to hypertension and other features of preeclampsia.^{17,18}

Gasotransmitters in Pregnancy

Nitric Oxide

NO is a potent vasodilator formed by the conversion of L-arginine to L-citrulline by one of the three NO synthase (NOS; Figure 1) isoforms. NO mainly acts through activation of its second messenger, cyclic guanosine monophosphate.⁸ Endothelial NOS (eNOS) is involved in regulation of peripheral vascular resistance during pregnancy.^{8,19} The biosynthesis of NO increases along with gestation (cGMP).⁸ This is reflected by increasing amounts of NO metabolite (nitrite/nitrate) levels in maternal plasma.⁸ cGMP is also increased in plasma and urine indicating an increased bioactivity of NO during pregnancy.⁸ In addition, plasma asymmetrical dimethylarginine (ADMA), a competitive inhibitor of NOS, is reduced.²⁰

In humans, eNOS and inducible NOS proteins are expressed by the fetal endothelium and villous trophoblasts. Inducible NOS is also expressed by Hofbauer cells (macrophages; Figure 2).²¹⁻²³ In the placental bed, inducible NOS and eNOS are expressed by interstitial and endovascular cytotrophoblasts.^{24,25} NO might be involved in trophoblast invasion and spiral artery remodeling because the ability of trophoblasts to remodel the spiral arteries relates to local NO production by interstitial trophoblasts.³ In addition, placentae from eNOS-deficient mice have spiral arteries with a retained smooth muscle cell layer.²⁶ NO may also be involved in fetal vascular function in the placenta because it is able to vasodilate fetoplacental vasculature in an isolated human placental cotyledon.⁴

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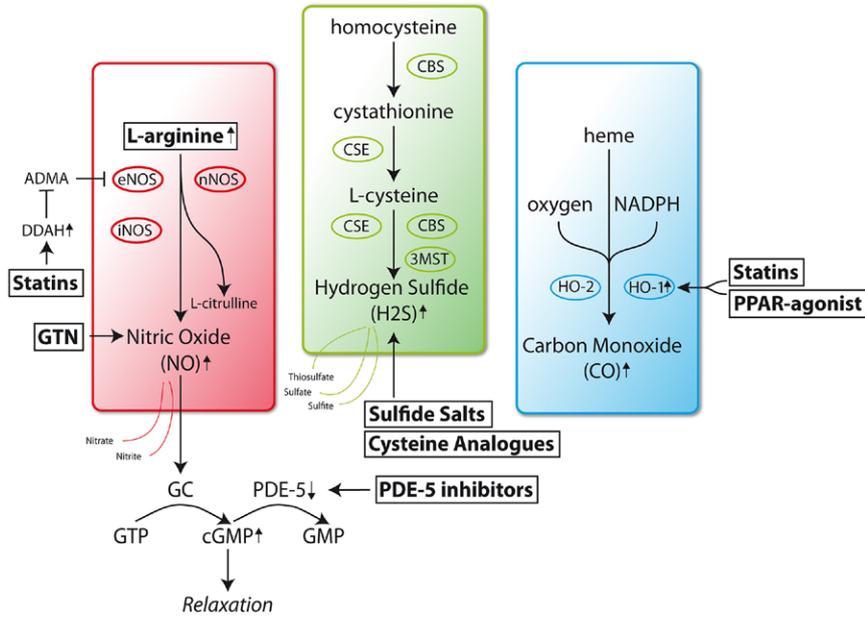


Figure 1. Schematic overview of the enzymatic production of NO, H₂S, and CO, and agents able to increase functionality or availability of gasotransmitters with potential therapeutic effect in pre-eclampsia. NO (left) is produced by 3 enzymes: neuronal NO synthase (nNOS), inducible NO synthase (iNOS), and endothelial NO synthase (eNOS) from L-arginine. Nitrate and nitrite are metabolites of NO production. H₂S (middle) is produced by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3MST) from homocysteine, cystathionine, and L-cysteine. Thiosulfate, sulfate, and sulfite are metabolites of H₂S. Right, The enzymatic production of CO, formed from heme by heme-oxygenase-1 (HO-1) and HO-2. ADMA indicates asymmetrical dimethylarginine; cGMP, cyclic guanosine monophosphate; DDAH, dimethylarginine dimethylaminohydrolase; GC, guanyl cyclase; GTN, glyceryl trinitrate; GTP, guanosine triphosphate; PDE-5, phosphodiesterase type-5; and PPAR, peroxisome proliferator-activated receptors.

Carbon Monoxide

CO is a gaseous vasodilator synthesized during the conversion of heme to biliverdin by the enzymes heme oxygenase-1 and heme oxygenase-2 (HO-1 and HO-2, respectively; Figure 1). CO protects against ischemia-reperfusion injury and inhibits inflammatory responses.²⁷ During pregnancy, the HO/CO system is thought to maintain maternal systemic vascular tone.⁵ In pregnant mice, increased HO-activity decreases peripheral

vascular resistance, although pregnant mice heterozygous knocked out for the HO-1 gene demonstrated increased diastolic blood pressure.^{5,28}

In the placenta, HO-1 and HO-2 are expressed in fetal endothelium and villous trophoblasts, whereas in the placental bed both enzymes are expressed by interstitial and endovascular trophoblasts (Figure 2).^{11,29,30} HO-1 is also expressed by leucocytes and other cells in the decidua.¹² In isolated

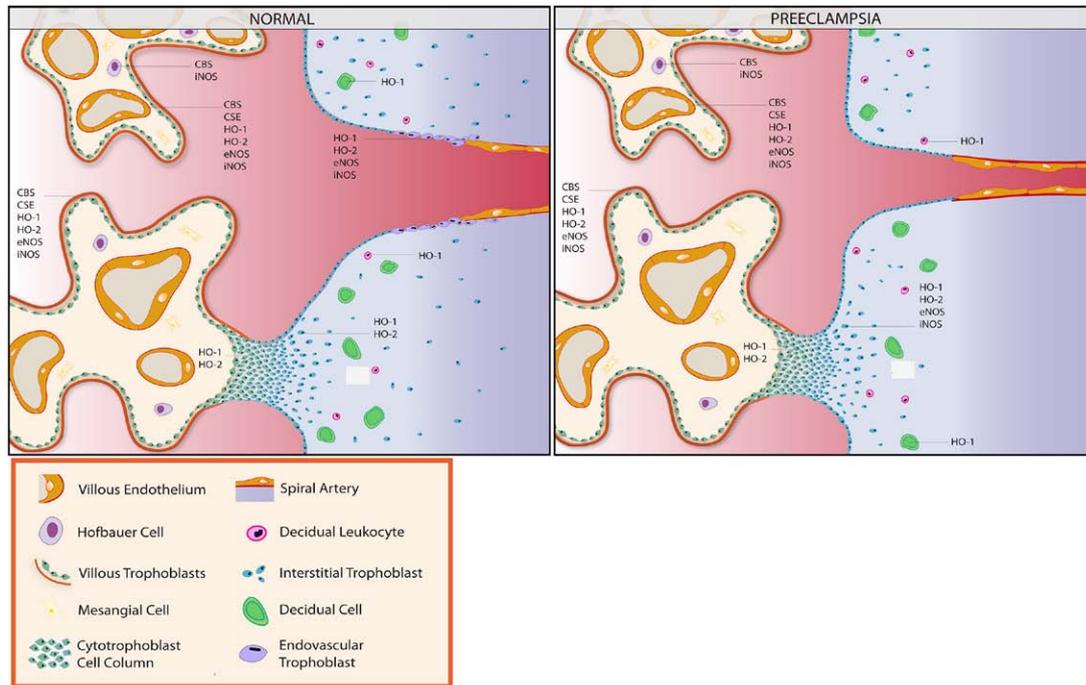


Figure 2. Gasotransmitter-producing enzyme expression in the human placenta. The enzymes are heterogeneously distributed throughout the human placenta.^{10-12,21-25,29,30,33} Healthy term placenta (left) and preeclamptic placenta (right). Cytotrophoblast invasion is shallow and fails to enter the maternal myometrium resulting in smaller and high-resistant spiral arteries. CBS indicates cystathionine-β-synthase; CSE, cystathionine-γ-lyase; eNOS, endothelial NO synthase; HO, heme oxygenase-1; HO-2, heme oxygenase-2; and iNOS, inducible NO synthase.

human placenta, CO decreases villous vascular tone and HO-inhibition causes dose-dependent constriction of the fetoplacental vasculature.^{7,30} In vitro, HO was found to stimulate trophoblast invasion.³¹

Hydrogen Sulfide

H₂S is produced by cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase from L-cysteine (Figure 1).² H₂S mediates vascular resistance and systemic blood pressure,³² has anti-inflammatory effects, promotes angiogenesis, and protects against ischemia-reperfusion injury.^{2,27} A precursor in the H₂S production pathway is homocysteine converted by CBS into cystathionine.

In human placenta, CBS is expressed by the fetal endothelium, Hofbauer cells, and villous trophoblasts, whereas CSE is expressed by the fetal endothelium and vascular smooth muscle cells (Figure 2).^{10,33} In the placenta, these enzymes are active (both rat and human) and are able to produce H₂S.⁶ The catalytic activity of placental CBS was also shown by incubation of human placental explants with increasing homocysteine concentrations leading to elevated CBS activity and increased cysteine in vitro.³⁴ In the human fetoplacental circulation, H₂S mediates vasodilatation in vitro via K_{ATP} channels and by interaction with NO.³³ CBS deficiency causes impaired decidualization in mice.³⁵

Gasotransmitters in Preeclampsia

The Table summarizes alterations and actions of the gasotransmitters and precursors in the 2 stages of preeclampsia both in the placenta and in the systemic circulation. The presence of alterations before the onset of preeclampsia, that is, in stage 1 of the disease, implies that alterations may be primarily and not because of the onset of preeclampsia.

Nitric Oxide

A recent meta-analysis showed that genetic variations in the eNOS gene contribute to an increased risk for preeclampsia.³⁶ Data on circulating NO in preeclampsia are inconsistent.⁸ The severity of the disease or gestational age at sampling

might explain these variations. However, concentrations of cGMP are consistently lower in preeclampsia indicating decreased NO bioactivity.⁸ This is in line with increasing plasma ADMA during and before the onset of preeclampsia as compared with healthy pregnancy.⁹

The placental protein expression of inducible NOS and eNOS is not different in preeclampsia versus control.^{22,23,37} However, the total activity of the enzymes is decreased in preeclamptic placenta.³⁸

The modulating effect of NO on the angiogenic balance might be important during preeclampsia. NO production in primary human trophoblasts increased placental growth factor and VEGF and decreased sFlt1 mRNA expression resulting in an enhanced proangiogenic environment in vitro.³⁹ In addition, lack of eNOS aggravates the sFlt1-induced preeclampsia-phenotype in mice.⁴⁰ Because NO production is impaired during preeclampsia, the pathway may be insufficient to counteract pathological events like the antiangiogenic state.

Carbon Monoxide

In the circulation of preeclamptic women, HO-1 enzyme levels are elevated.¹² However, CO concentrations in end-tidal breath of preeclamptic women are lower compared with controls.⁴¹ The difference might be explained by decreased HO-1 activity during preeclampsia. Increased CO consumption, reflected by HO-1 induction and lower secretion of CO by breathing, can also explain this difference.

Placental gene and protein expression of HO-2 is unaltered during preeclampsia.^{11,42,43} However, HO-1 expression is affected by preeclampsia: during the first trimester, that is, in stage 1 of the disease, HO-1 gene expression in chorionic villi is lower in preeclampsia.⁴⁴ However, during preeclampsia, data on placental HO-1 expression and activity are conflicting.^{11,42,43,45} In decidual leukocytes and other decidual cells, HO-1 expression is increased.¹² Because the decidua is one of the major sources of oxidative and inflammatory stress products in preeclampsia, increased decidual expression of the cytoprotective protein HO-1 might act as a protective mechanism during preeclampsia.¹²

The HO/CO-system might influence the angiogenic balance in preeclampsia. Mice heterozygous for the HO-1 gene show

Table. Overview of Alterations and Actions of Gasotransmitters and Related Pathways in Preeclampsia

Trophoblast Studies (STAGE I)		Alterations Before the Onset of Preeclampsia (Stage 1)		Alterations During Preeclampsia (Stage 2)			
		Maternal Circulation		Placenta		Maternal Circulation	
NO	Stimulates trophoblast invasion* ³	ADMA eNOS	Increased ⁹ Increased genetic variations ³⁶	eNOS iNOS	Decreased activity ³⁸ Decreased activity ³⁸	Nitrite/nitrate cGMP	Inconsistent data ⁸ Decreased ⁸
CO	Stimulates trophoblast invasion† ³¹	HO-1	Decreased ⁴⁴	HO-1 HO-1 HO-2	Inconsistent data ^{11,42,43,45} Increased in decidua ⁴⁵ Unaltered ^{11,42,43}	HO-1 CO	Increased ¹² Decreased ⁴¹
H ₂ S	PAG (inhibition of CSE) reduces trophoblast invasion ⁴⁹	Homocysteine	Increased ⁴⁸	CBS CSE	Inconsistent data ^{10,33,49} Inconsistent data ^{10,33,49}	H ₂ S	Decreased ⁴⁹

ADMA indicates asymmetrical dimethylarginine; CBS, cystathionine-β-synthase; cGMP, guanosine monophosphate; CO, carbon monoxide; CSE, cystathionine-γ-lyase; eNOS, endothelial NO synthase; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; H₂S, hydrogen sulfide; iNOS, inducible NO synthase; and PAG, DL-propargylglycine.

*Animal study.

†In vitro trophoblasts.

morphological changes in the placenta similar to preeclampsia and elevated diastolic blood pressure and plasma sFlt1.^{28,46} The relation between the CO/HO system and sFlt1 was further shown in in vitro experiments: overexpression of HO-1 in endothelial cells decreased the production of sFlt1 and sEng, whereas HO-1 knockdown potentiates sFlt1 and sEng release from both endothelial cells and placental villous explants.⁴⁷

Hydrogen Sulfide

First reports on the H₂S pathway showed increased amounts of plasma homocysteine during and before the onset of preeclampsia.⁴⁸ This could be because of decreased CBS or CSE activity in the placenta.^{10,33,49} However, studies on villous placental expression of CBS and CSE in preeclampsia are conflicting.^{10,33} Although 2 reports showed decreased CSE mRNA and protein levels in placentae of patients with preeclampsia,^{33,49} we found no differences in CSE expression but a decreased CBS expression.¹⁰ Differences in severity of the disease may play a role in these different results. Interestingly, miRNA-21, which downregulates CSE, is elevated in the abnormal Doppler placentae.³³ In vitro experiments with human placentae show that H₂S production increases under hypoxic conditions.⁶

A potential role for these enzymes in the pathophysiology of preeclampsia has been shown by in vitro experiments, in which downregulation of CSE was associated with alterations in angiogenic factors (increased sFlt1 and sEng and decreased placental growth factor).⁴⁹ Although pregnant CBS-deficient mice exhibit hyperhomocysteinemia which is associated with blunted endothelial-dependent vessel relaxation.⁵⁰ Another pathophysiological role for decreased H₂S during preeclampsia might be its ability to alter the angiogenic balance. H₂S increases angiogenesis by upregulation of VEGF and by direct action on the VEGFR2 receptor.^{51,52} Also, H₂S-stimulated ischemic vascular growth is dependent on augmented expression and activity of VEGF.⁵³ H₂S also has an anti-inflammatory potential,²⁷ and decreased H₂S might be involved in the activated inflammatory response in preeclampsia. H₂S is able to downregulate several proinflammatory cytokines, such as interleukin-6 and tumor necrosis factor- α , and modulate leukocyte adhesion and infiltration.⁵⁴ Finally, H₂S is a potent antioxidant and a scavenger of reactive oxygen species which are abundantly present during preeclampsia.⁵⁴

Gasotransmitters in Preeclampsia: A Therapeutic Potential?

During preeclampsia, the functionality of gasotransmitters is aberrant. Therefore, factors able to increase functionality or availability of gasotransmitters may be beneficial as therapeutic agents in (Figure 1).

Nitric Oxide

NO drugs that enhance NO availability are widely available. In 2007, a Cochrane review concluded that there was insufficient evidence to use the NO-donor glyceryl trinitrate and L-arginine, to prevent, or to treat preeclampsia.⁵⁵ However, more recent studies showed that these drugs might have therapeutic potential in preeclampsia. In vitro data showed that glyceryl trinitrate protects chorionic villi from ischemia-reperfusion injury, although it inhibits release of sFlt1 and

sEng.^{56,57} Although this drug is applicable during pregnancy, the severe headaches that are reported avert its use.⁵⁵ L-Arginine attenuates hypertension in rat models with reduced uterine perfusion pressure and sFlt1 overexpression.^{58,59} In human pregnancy, L-arginine did not lower blood pressure in women with pre-existent hypertension, but supplementation resulted in less need for additional antihypertensive medication.⁶⁰ The combination of L-arginine and antioxidant vitamins (C, E) prolonged the latency to develop preeclampsia in a high-risk population.⁶¹

Phosphodiesterase type-5 inhibitors are novel in the preeclampsia field and potentiate the effects of NO by preventing cGMP degradation. In the L-NAME (N^G-nitro-L-arginine methyl ester) rat model for preeclampsia, Sildenafil supplementation from day 7 of gestation decreased fetal mortality, lowered (but not normalized) blood pressure, and reduced albuminuria, sFlt1, and sEng.^{62,63} When administered from day 0 of gestation, Sildenafil prevented hypertension and proteinuria⁶⁴ suggesting that timing of treatment in pregnancy is important. This therapeutic potential of Sildenafil clearly calls for a randomized controlled trial in which Sildenafil is administered in a high-risk population before onset of preeclampsia.

Because increased concentrations of ADMA can be measured before clinical manifestations of preeclampsia, agents decreasing ADMA could have therapeutic potential.⁹ ADMA levels can be reduced, by agents enhancing activity or expression of dimethylarginine dimethylaminohydrolase. Pravastatin increases dimethylarginine dimethylaminohydrolase in vitro.⁶⁵ Indeed, in mice with sFlt1-induced preeclampsia, pravastatin increased vascular eNOS and ameliorated hypertension, proteinuria, and glomerular endotheliosis.^{65,66} Moreover, pravastatin was able to antagonize sFlt1 by upregulation of placental growth factor.⁶⁵ The first clinical trial with pravastatin in women with preeclampsia (Statins to Ameliorate early onset preeclampsia [StAmP] trial) is currently being executed.

Interestingly, the therapeutic effect of NO its metabolite nitrate is recently described. It was shown that nitrite protects against ischemic renal injury and that nitrite in beetroot attenuates human hypertension.^{67,68} Therefore, studying the effect of nitrite in preeclampsia would be of high value.

Being widely available and suitable for clinical use during pregnancy, the therapeutic potential of NO-based agents seems promising. However, evidence is based on animal experiments; results of randomized controlled trials are lacking.

Carbon Monoxide

CO agents able to increase HO expression, and consequently CO production, might be beneficial in preeclampsia. This is in line with the well-known effect of smoking in pregnancy: the incidence of preeclampsia in smokers is reduced by 32%. This may be partly attributed to increased CO.⁶⁹ Induction of HO-1, or delivery of its bioactive metabolites CO and bilirubin, downregulated sFlt1 and reduced oxidative stress in placental explants in vitro and attenuated hypertension in preeclamptic rats.^{11,70,71}

As mentioned previously, pravastatin influences the ADMA/NOS/NO pathway during pregnancy. Statins can also exert anti-inflammatory and antiproliferative effects through HO-1 induction.⁴⁷ Simvastatin improves symptoms of preeclampsia

by upregulation of HO-1 and downregulation of sFlt1 expression in healthy term placental explants.⁴⁷

Another compound capable of inducing HO-1 is rosiglitazone, a peroxisome proliferator-activated receptor (PPAR)-agonist. PPARs play a role in trophoblast differentiation, and reduced placental expression of PPAR is described in preeclampsia.^{72,73} In models of cardiovascular disease, activation of PPAR restores vascular structure and endothelial function.⁷⁴ Its beneficial effects are mediated by upregulation of HO-1.⁷⁴ In experimental preeclampsia, the PPAR-agonist rosiglitazone ameliorates hypertension and endothelial dysfunction in a HO-1-dependent pathway.⁷⁴

In conclusion, agents capable of HO induction are beneficial as therapeutic agents in experimental preeclampsia. Statins and PPAR-agonists are HO-inducing agents and ameliorate hypertension, restore endothelial function, and lower sFlt1 in experimental preeclampsia. Unfortunately, human data are lacking.

Hydrogen Sulfide

H₂S-based therapies are emerging in the field of cardiovascular diseases, and H₂S-releasing compounds are developed for clinical use. These compounds can be divided into 2 groups: sulfide salts and synthetic H₂S-donors.⁷⁵ Sulfide salts include sodium hydrosulfide and sodium sulfide. Intravenous administration of sodium sulfide in humans increased H₂S levels, and the compound is now tested in phase II trials for cardiac ischemia-reperfusion injury (clinicaltrials.gov).⁷⁶ Synthetic H₂S-donors mainly consist of cysteine analogues, such as s-allyl cysteine, S-propyl cysteine, and n-acetyl cysteine, which all increase H₂S production and decrease ischemia-reperfusion injury in a rat models.⁷⁵ Although beneficial effects of s-allyl cysteine on placental oxidative stress were shown in vitro, oral n-acetyl cysteine administration to women with preeclampsia did not alter maternal and fetal outcomes.⁷⁷ A recent experimental study showed that preeclampsia symptoms (including alterations in angiogenic factors) in mice, induced by CSE-inhibition by DL-propargylglycine, were restored by a slow releasing H₂S compound (GYY4137).⁴⁹

Taken together, H₂S-releasing compounds are available and clinically tested. However, too few studies on their therapeutic potential during preeclampsia were performed to draw any conclusions.

Conclusions

NO, CO, and H₂S have a high potential for therapeutic intervention in preeclampsia. Their shared vasoactive properties, anti-inflammatory functions, reactive oxygen species scavenging capacities, and angiogenic potential are shown to be cardinal during pregnancy and in the development of preeclampsia. Clinical trials showing definite therapeutic potential and safety of gasotransmitters in preeclampsia are warranted. We firmly believe that these volatile substances could well be a solution for the continuing therapeutic dilemma of preeclampsia.

Disclosures

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