Sulfane Sustains Vascular Health
Insights Into Cystathionine γ–Lyase Function
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Hydrogen sulfide (H2S) is widely known as a pungent toxic gas that has plagued humanity in various environmental conditions for centuries. However, H2S, along with nitric oxide (NO) and carbon monoxide (CO), is now recognized to be an important biological gasotransmitter that was previously believed to simply be an environmental toxin.1 H2S can be produced in the mammalian body by 3 enzymes: cystathionine γ-lyase (CSE), cystathionine β synthase, and 3-mercaptopropionate sulftransferase from the substrates cystathionine, homocysteine, cysteine, and mercaptopropionate. Recently, the physiological importance of H2S in the cardiovascular system, particularly vascular growth and inflammatory regulation, has been recognized; however, information on the importance of endogenous H2S synthesis pathways and identification of critical enzymes has been less clear.2 In this issue of Circulation, 2 complementary articles examining endogenous H2S production and metabolism functions provide important insight into the role of CSE and H2S bioavailability for vascular pathophysiological responses during preeclampsia and atherosclerosis. The first article by Wang et al3 emphasizes the emergence of an important role for H2S in regulating placental vasculature dysfunction during preeclampsia by altering placental growth factor, soluble Flt-1 (sFlt-1), and soluble endoglin (sEng) levels. The second article by Mani4 and colleagues provides important insight into the role of endogenous H2S production in modulating angiogenic imbalance in the placenta.5

Preeclampsia
Preeclampsia is a pregnancy-related vascular disorder characterized by hypertension, proteinuria, and peripheral edema. Although the exact cause of preeclampsia is unknown, possible causes include systemic endothelial dysfunction and impaired vascular growth and remodeling in the placenta.5 Human placenta expresses vascular endothelial growth factor and its receptor (flt-1). According to the angiogenic imbalance hypothesis, loss of vascular endothelial growth factor activity causes preeclampsia as a result of an elevation of sFlt-1, an endogenous inhibitor of vascular endothelial growth factor. Evidence supports this hypothesis in that maternal circulating levels of sFlt-1 and sEng (a cleavage product of transforming growth factor-β1) are elevated and placental growth factor levels are low in women who develop preeclampsia.6 Continuously elevated levels of sFlt-1 and sEng ultimately lead to maternal endothelial dysfunction and impaired angiogenesis in the placenta.

CSE and H2S in Preeclampsia
H2S has potent effects on physiological responses such as angiogenesis, inflammation, vasodilation, and modulation of oxidative and redox stress. In regard to the angiogenic effect of H2S, evidence suggests that H2S promotes angiogenesis via stimulating PI3K/Akt or mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathways.10,11 Moreover, H2S can alter angiogenic activity via crosstalk with NO through enzymatic (eg, endothelial NO synthase) or non-enzymatic pathways such as conversion of nitrite to NO.12,13 It is well known that among the 3 H2S-producing enzymes, CSE and cystathionine β synthase are present predominantly in human intrauterine tissue and placenta. However, the role of CSE/H2S in placental abnormalities or preeclampsia has been unclear. The groundbreaking study by Wang et al lays the foundation for understanding the role of CSE/H2S during preeclampsia. They found that H2S levels are reduced in plasma of pregnant women with preeclampsia and that CSE enzyme expression is reduced in preeclamptic placental tissue. Additionally, they provide clear evidence that circulating placental growth factor levels are reduced in women with preeclampsia associated with dysregulation of CSE/H2S signaling pathway. These findings are associated with CSE/H2S-mediated prevention of release of sFlt-1 and sEng. Importantly, animal studies inhibiting CSE activity in pregnant mice recapitulated key features of preeclampsia, including hypertension, elevation of sFlt-1 and sEng, defective placental vascularization, and arrest of fetal growth, which

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were reversed by exogenous H$_2$S therapy. Thus, this study shows that H$_2$S rescues placental vasculature abnormalities and ameliorates hypertension and fetal growth restriction in the mouse placenta. These discoveries open new possibilities for preeclampsia diagnosis and therapeutics.

Although Wang et al have shown compelling evidence of a crucial role for CSE/H$_2$S during preeclampsia, many questions await further study. Why is CSE/CSE expression reduced during preeclampsia, and what are the effects on alternative H$_2$S-generating enzymes such as cystathionine $\beta$ synthase and 3-mercaptopyruvate sulfurtransferase? What is the precise concentration of H$_2$S needed to maintain placental vascular health, and are different biochemical pools of H$_2$S (eg, free, acid labile, and bound sulfane sulfur) altered during preeclampsia? Using analytical measurement techniques could address these important questions and would provide key information necessary to move therapeutic studies forward. Thus, further study is needed to understand the regulation of expression and activity and the substrate bioavailability that may also contribute to H$_2$S regulation of preeclampsia. Finally, there could be interaction with other factors such as NO, CO, and vascular endothelial growth factor that are known to be involved in preeclampsia pathophysiology. It is possible that interactions between these metabolic mediators could collectively participate in pathological mechanisms that await further investigation.

**Atherosclerosis**

Atherosclerosis is a chronic and complex inflammatory process involving different cellular and molecular signaling pathways that leads to fat accumulation, plaque formation, and stenosis of the arterial lumen. The gaseous mediators NO, CO, and H$_2$S generated within the vasculature have been implicated in modulating vascular functions involved during atherogenesis. It is well known that dysregulation of NO production in the vasculature promotes atherosclerosis formation as a result of impaired $\omega$-arginine utilization, inactivity of endothelial or inducible NO synthase, and decreased NO bioavailability. Similarly, decreased production of CO resulting from reduced expression of heme oxygenase-1 stimulates atherosclerotic plaque formation. However, the role of H$_2$S in atherosclerosis is a novel finding that has been explored recently. Past research suggested that CSE expression and activity and subsequent H$_2$S production were reduced during balloon-mediated injury and neointima development. Importantly, H$_2$S can inhibit vascular smooth muscle cell proliferation, neointimal hyperplasia, and atherosclerotic plaque size via inhibition of the mitogen-activated protein kinase/extracellular signal-regulated kinase/caspase-3 signaling pathway. However, the importance of endogenous enzyme synthesis mechanisms for H$_2$S production during atherogenesis remained unclear.

**CSE and H$_2$S in Atherosclerosis**

In the study by Mani et al, the authors provide important new information on the role of CSE regulation of lipid metabolism and regulation of atherosclerosis formation. Using an atherogenic diet, the authors revealed that genetic deficiency of CSE in mice resulted in increases in plasma total and low-density lipoprotein cholesterol and atherosclerotic lesions, which were rectified by exogenous H$_2$S therapy. Importantly, hypertension caused by CSE mutation did not significantly contribute to enhanced atherogenesis. It was also shown that CSE-apolipoprotein E double-knockout mice had a larger atherosclerotic lesion area that could be attenuated with exogenous H$_2$S.

With regard to possible molecular mechanisms, the authors found that CSE knockout augmented oxidative stress during atherosclerosis (ie, lower levels of glutathione and superoxide dismutase and higher levels of malondialdehyde), which was corrected by exogenous antioxidant N-acetyl cysteine therapy. In addition, vascular smooth muscle cell glutathione peroxidase and glutathione reductase protein levels were reduced and reactive oxygen species production was increased in CSE knockout mice, which were reversed by H$_2$S therapy, indicating a role of CSE dependent of H$_2$S in modulating redox stress defense during atherosclerosis. It was further observed that nuclear factor-kB–mediated intercellular adhesion molecule-1 expression was significantly increased in smooth muscle cells of CSE knockout mice compared with WT mice and that exogenous H$_2$S attenuated this response. Thus, this study clearly identified the importance of endogenous H$_2$S production via CSE in regulating atherogenesis, highlighting the possibility of therapeutic approaches aimed at modulating enzyme expression or function during atherosclerosis.

Although the authors provide clear proof of concept for an important role of CSE/H$_2$S during atherosclerosis, many questions remain unanswered. What are the levels of plasma and tissue H$_2$S needed to guard against atherogenic mechanisms? Is H$_2$S bioavailability differentially distributed in various biochemical forms throughout the vasculature itself, thereby rendering regions of the vascular tree susceptible to atherogenesis? Additionally, studies focused on determining the function and
regulation of CSE expression in different cell types involved in atherosclerosis are necessary to better understand the pathophysiological processes most affected by H₂S metabolism. Finally, given the recent appreciation of H₂S-NOS pathway interactions, it is possible that novel crosstalk mechanisms may be involved in regulating inflammation during atherosclerosis.  

Conclusions

Results from these studies expand our understanding of the importance of CSE/H₂S signaling pathway in regulating vascular responses in diseases that primarily involve them. Together, these results begin to paint a picture in which CSE and H₂S bioavailability regulates vascular health to control different pathological responses, as illustrated in the Figure. The notion that H₂S may be important for vascular health and function further suggests that therapeutic attempts to modulate synthesis enzyme pathways or bioavailability may be beneficial for diseases with a foundation of vascular pathology. However, additional detailed studies on H₂S bioavailability and its protective mechanisms are needed to better understand the role of this gaseous mediator. The realization of the CSE/H₂S synthesis paradigm for vascular pathologies provides important new information that could reveal novel therapeutic approaches.

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References


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