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Homocysteine in renovascular complications: hydrogen sulfide is a modulator and plausible anaerobic ATP generator

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Abstract

Homocysteine (Hcy) is a non-protein amino acid derived from dietary methionine. High levels of Hcy, known as hyperhomocysteinemia (HHcy) is known to cause vascular complications. In the mammalian tissue, Hcy is metabolized by transsulfuration enzymes to produce hydrogen sulfide (H_2S). H_2S, a pungent smelling gas was previously known for its toxic effects in the central nervous system, recent studies however has revealed protective effects in a variety of diseases including hypertension, diabetes, inflammation, atherosclerosis, and renal disease progression and failure. Interestingly, under stress conditions including hypoxia, H_2S can reduce metabolic demand and also act as a substrate for ATP production. This review highlights some of the recent advances in H_2S research as a potential therapeutic agent targeting renovascular diseases associated with HHcy.

Keywords

Renal remodeling; homocysteine; hydrogen sulfide; extracellular matrix; matrix metalloproteinase; inflammation; mitophagy; hypertension

1. Introduction

Progressive decline of renal function in chronic kidney diseases such as glomerulosclerosis and tubular-interstitial fibrosis impairs the ability of kidney to excrete waste products and maintain water and electrolyte balance. Renal microvascular endothelial injury, vessel calcification and remodeling can increase vascular resistance causing elevation of blood pressure [1; 2],. Clinical data suggests an association between systolic hypertension, renal dysfunction and high levels of plasma homocysteine (Hcy). There is an inverse relationship between plasma Hcy levels and progressive decline in renal function.

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In the body, Hcy is metabolized by two distinct pathways: 1) remethylation back to its precursor methionine, and 2) transsulfuration to form H_2S. Since the original discovery of H_2S biogenesis, it has gained substantial interest in the research community for determining its role in health and disease. Current evidence suggests that H_2S regulates a number of physiological processes including but not limited to synaptic transmission, vasorelaxation, pro- and anti-inflammatory effects, angiogenesis, smooth muscle cell proliferation and migration, and autophagy. In hyperhomocysteinemia (HHcy), decreased H2S has been linked to disease progression and morbidity. HHcy and impaired H2S formation is commonly seen in patients with cirrhosis [3]. Further, HHcy in cirrhosis has been shown to cause endothelial dysfunction in rats which was reversed following H2S treatment [3]. In a rat model of HHcy, Wei el al demonstrated marked increase in endoplasmic reticulum (ER) stress in cardiomyocytes and reduction in endogenous H_2S production, whereas H_2S supplementation decreased the expression of ER stress-associated proteins [4]. Increased Hcy levels and decreased H_2S production has been reported in patients undergoing hemodialysis for uraemia [5]. Also, low levels of H_2S have been demonstrated in HHcy-induced hypertension wherein both endogenous and exogenous H_2S was shown to mitigate high blood pressure suggesting a key role in blood pressure regulation [6]. Taken together, the above studies suggest an inverse relationship between Hcy and H_2S in diverse pathologies.

In this review we discuss recent developments on Hcy handling, particularly its synthesis, accumulation, and metabolism in renal vasculature. Furthermore, in light of present literature the roles of H_2S are highlighted as a molecule which mitigates renovascular complications and hypertension.

2. Hcy biosynthesis and accumulation

Hcy is a non-protein α-amino acid derived from methionine and is a homologue of amino acid cysteine, differing by an additional methylene group. The normal plasma Hcy levels in humans range from 5–15 μmol/L. In rare inborn errors of metabolism, levels >100 μmol/L have been reported [7]. Based on the plasma concentration, HHcy is categorized into three groups, mild (>15 μmol/L to italic> 30 μmol/L), moderate (> 30 μmol/L to bold> 100 μmol/L) and severe (>100 μmol/L) [8]. A number of studies suggest moderate HHcy as an independent risk factor for vascular diseases including coronary artery disease and venous thromboembolism [9; 10]. To aid US Preventive Services Task Force for finding novel risk factors for coronary heart disease (CHD), a meta-analysis was performed, and the analysis suggests that each increment of Hcy level by 5 μmol/L increases the risk of CHD events by approximately 20 percent [11]. This underlines the clinical significance of HHcy.

There is however no direct diet source of Hcy; instead it is biosynthesized from methionine. Hcy biosynthesis, accumulation and metabolism in the body depend on many factors and several pathways contribute to regulate plasma Hcy levels. Five major pathways are involved in this process, a) methylation reaction, b) remethylation pathway, c) renal mechanism via volume retention, d) transsulfuration pathway, and e) protein-energy malnutrition (Figure 1). Among the pathways, methylation reaction, renal volume retention and protein-energy malnutrition increase plasma Hcy levels; whereas, remethylation and

transsulfuration decrease its level. In cells, methionine by condensation reaction with ATP forms methyl donor, *S*-adenosylmethionine (SAM) (Figure 1). In the methylation reaction, SAM is transformed into SAH (*S*-adenosylhomocysteine) by donating its methyl group to other substrates, and in subsequent reversible reaction SAM produces Hcy. Hcy is converted back to methionine through folate and vitamin B_{12} remethylation pathway. Excess Hcy in circulation is cleared by the kidney and liver. The kidney is capable of filtering as well as metabolizing Hcy [12]. Although the molecular mass of Hcy (135 D) is within the filtration range of glomeruli, major portion of filtered Hcy is absorbed by tubular uptake [12]. In addition, kidney contains transsulfuration enzymes CBS, CSE, 3MST and CAT as well as remethylation enzymes [13; 14; 15; 16]. Therefore, renal pathways of Hcy handling largely depend on filtration, reabsorption and metabolism (remethylation and transsulfuration) ability of kidney [17]. In chronic kidney diseases (CKD) reduced glomerular filtration thus increases plasma Hcy accumulation [12]. In addition, dysregulation in the transsulfuration enzymes further increases plasma Hcy levels [18; 19; 20]. Similar to kidney, the liver remethylates Hcy back to methionine through remethylation (Figure 1) [12; 21], and can also metabolize it by transsulfuration pathway [22; 23]. A derangement in either of the pathways can therefore result in abnormal increase in Hcy. In addition, protein-energy malnutrition is also reported to increase plasma Hcy levels (Figure 1) [24].

3. Hcy pathobiology in kidney diseases

HHcy is a recognized risk factor for vascular diseases [25; 26]. Individuals with advanced CKD [27] and patients undergoing hemodialysis [28] are reported to have high levels of plasma Hcy, which may further contribute to renovascular injury leading to a vicious cycle [29]. Renal injury has been reported in an experimental weanling rat model of HHcy [29; 30] which is consistent with our own recent findings in mice [19; 31]. A recent systemic review and meta-analysis reported that glomerular filtration rate inversely correlates with Hcy levels with HHcy prevalence of 36–89% in patients with CKD, 70–75% in patients with viable kidney transplants and 85–100% in end-stage renal disease [32]. In vivo studies have demonstrated that HHcy can increase blood pressure by causing arteriolar constriction, arterial stiffness, endothelial damage, and increased sodium absorption [33]. The underlying mechanisms include: a) oxidative stress, b) smooth muscle cell proliferation, c) inflammation, d) autophagy, e) extracellular matrix remodeling, f) formation of Hcy-thiolactone, and g) protein homocysteinylation. Protein modification by homocysteinylation has been implicated in the development of atherogenesis and atherothrombosis [34; 35]. Indeed patients deficient in cystathionine β-synthase (CBS) were shown to have increased levels of prothrombotic N-Hcy-fibrinogen increasing their risk for thrombotic events [35]. In addition, Hcy may homocysteinylate endothelial nitric oxide synthase (eNOS), resulting in decreased nitric oxide (NO) production by endothelial cells including renal vessels. This possibility however is yet to be confirmed.

4. Metabolism of Hcy and formation of H_2S

Metabolism of Hcy occurs by two distinct pathways: 1) remethylation wherein Hcy receives a methyl group to form its precursor molecule methionine, and 2) transsulfuration pathway to form cysteine (Figure 1). In the remethylation process, Hcy acquires a methyl group from

N-5-tetrahydrafolate in the presence of vitamin B12 catalyzed by homocysteine methyltransferase (HMT) and occurs in all tissues. In the alternate pathway occurring exclusively in the liver, Betaine HMT converts Betaine to dimethylglycine releasing a methyl group which couples with Hcy to form methionine [36].

In the transsulfuration pathway, Hcy condenses with serine to from cystathionine (Figure 2). A pyridoxal-5′-phosphate (PLP)-containing enzyme, cystathionine β-synthase (CBS) catalyzes this reaction where vitamin B_6 acts as a co-factor. Cystathionine is further hydrolyzed by a second PLP enzyme, cystathionine γ-lyase (CSE) to form cysteine. Cysteine is the main precursor of endogenous H_2S formation; thus, Hcy and cysteine synthesize H_2S by cytosolic enzymes CBS and CSE, or by the sequential activity of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur transferase (3MST) in both the cytosol and mitochondria (Figure 2). H_2S synthesis by other mechanisms independent of Hcy has been summarized recently in a review article by Olson et al [37].

5. Physiology of H_2S in the kidney

H_2S has long been known for its neurotoxicity and as an environmental hazard. The ground breaking work by Abe and Kimura in 1996 however demonstrated for the first time that the hippocampus produces H_2S, which functions as a neuromodulator [38]. A subsequent study by Kimura H reported the role of H_2S as an endogenous smooth muscle relaxant suggesting possible regulation of vascular tone [39]. Subsequent studies by other researchers documented that endogenous H_2S has a variety of physiological functions and a decrease in its production was involved in diverse pathological processes, such as oxidative stress, vascular dysfunction, inflammation, neurodegenerative diseases, apoptosis, autophagy, atherosclerosis, hypertension and diabetes [40; 41; 42; 43; 44; 45]. These discoveries further stimulated research into its development as a potential therapeutic agent in diseases attributed to diminished H_2S synthesis [46].

In the kidney, H_2S is reported in a wide array of physiological functions. For example, Holwerda et al reported that H_2S attenuated hypertension and renal damage by upregulating VEGF [47]. In an rat model of unilateral ureteral obstruction (UO), exogenous H_2S donor inhibited renal fibrosis by attenuating the production of collagen and other extracellular matrix proteins [48]. In addition, H_2S attenuated inflammation by decreasing the expression of inflammatory cytokines and recruitment of macrophages [48]. In another study involving UO model, treatment with with sodium hydrogen sulfide reduced oxidative stress by preserving catalases such as CuZnSOD and MnSOD, and glutathione level [49]. Although these studies did not measure Hcy levels in UO model, decreased levels of H_2S were reported in both studies which are consistent with our previous finding in renal pathologies associated diminished H_2S and HHcy [18; 19]. Below, we review the beneficial effects of H_2S in renal pathology associated with HHcy. Of note, in a recent review Snijder et al summarized in depth on how H_2S, along with other two gasotransmitters, NO and carbon monoxide (CO), interact in renal transplantation to exert cytoprotection and reduction of tissue injury in transplanted organ [50].

6. Roles of H_2S in Hcy-mediated renovascular pathophysiology

6.1. Normal physiology of renal vasculature and gasotransmitters

Kidney is the filtration unit in vertebrate animals and comprises two major parts: cortex and medulla. The functional unit of kidney is nephron and is grossly divided into two parts; a) renal corpuscle located in the cortex and b) renal tubule that passes from the cortex into the medulla. The major function of the kidney is filtration, reabsorption of glucose, sodium, potassium and other solutes, secretion of hormones and maintaining fluid homeostasis in the body. Endogenous gaseous transmitters, NO, CO and H_2S, play immense role in maintaining normal physiological function of kidney. NO is produced by nitric oxide synthase (NOS) and the kidney expresses all three isoforms of NOS, i.e eNOS, nNOS and iNOS [51; 52]. In the kidney NO regulates renal hemodynamics, modulates fluid and electrolyte transport, and may also help minimize renal injury [53]. However, NO generated from iNOS may exacerbates renal injury in concert with oxidative-redox state particularly in the presence of super oxide due to the formation of peroxynitrite [54].

In the kidney, CO is generated during heme degradation by the enzymes heme oxygenase – 1 and -2 (HO-1 and HO-2) or fatty acid oxidation [55]. Studies have demonstrated that CO is a natural vasoprotector against excessive vasoconstriction and promotes natriuresis [55]. H_2S is the third gaseous molecule in the group and its production in the kidney is summarized in section 5 above. All the gasotransmitters above have similar physiological effects such as vasodilatory, anti-oxidant, anti-apoptotic, anti-inflammatory, and angiogenic properties through an array of inter- and intracellular signaling cascades [56] however, their mode of action and regulatory function may differ from each other [57]. In addition, recent literature suggests significant crosstalk between these gasotransmitters for synergism and normal physiology [58; 59]. In the following section, we discuss the physiological functions of the gasotransmitters in the kidney in relation to HHcy with an emphasis for using H_2S as a potential therapeutic agent.

6.2. Oxidative stress and endothelial dysfunction

A vast majority of literature suggest that Hcy causes oxidative injury to the endothelial cells (EC) [18; 60; 61]. Due to the presence of the highly reactive sulfhydryl group, Hcy can undergo auto-oxidation to generate oxygen radicals [62]. In circulation, the thiol group undergoes rapid metal-catalyzed auto-oxidation leading to generation of super oxide and hydrogen peroxide [60]. In addition to generating oxidant stress, Hcy has indirect effects on vascular redox status by diminishing the expression and activity of anti-oxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (PGx) [62; 63]. The endothelial cells which line the vessel walls are the primary target of these radicals. Since eNOS derived NO is the primary vasorelaxation factor under physiological conditions, the cells are deprived of NO following endothelial injury [64], leading to impaired endothelial function [65; 66]. In a transgenic CBS–deficient mice model Cheng et al reported that HHcy impairs NO– and endothelium-derived hyperpolarizing factor (EDHF)–mediated endothelium-dependent relaxation of small mesenteric arteries (SMAs) [67]. Hcy is also known to promote oxidation of eNOS cofactor, tetrahydro-L-biopterin (BH_4), resulting in BH_4-eNOS uncoupling and diminished NO production [68; 69; 70; 71]. Asymmetric dimethylarginine

(ADMA) is an endogenous inhibitor of NO synthase [72], and Hcy is reported to increase ADMA and thus decrease NO production [73]. In contrast to decreased eNOS-derived NO during HHcy, the activity of neural NOS (nNOS) and inducible NOS (iNOS) is increased by HHcy [74; 75]. This paradox, although presumed to have beneficial effects by vasorelaxation, the increase of NO in a highly reactive HHcy-induced oxidative environment can in fact lead to the formation of more potent peroxynitrite radical (ONOO-), known as reactive nitrogen species (RNS) [68]. This can lead to nitration of tyrosine residues in proteins thus impairing their function [76].

Evidence from animal models of HHcy suggest that endothelial dysfunction is largely due to oxidative stress and decreased bioavailability of NO [77]. A decrease in the production and circulation of H_2S can further contribute to endothelial-dependent impaired vasorelaxation in these models [18; 19; 20; 43]. This mechanism is partly mediated by interaction between H_2S and NO. Both H_2S and NO can engage in covalent reactions with proteins thereby temporarily modulating protein structure and function. H_2S reacts with Cys residues of the target protein through the formation of persulfide (-SSH) bond and this modification is known as protein *S*-sulfhydration [78]. Similarly, NO predominantly binds to sulfhydryl groups (-SH) of Cys residues of target protein, and the process is termed as protein *S*-nitrosylation [79]. It is also reported that H_2S functions in concert with NO forming nitrosothiol (RSNO) [80] or reacts with *S*-nitrosothiols to form thionitrous acid (HSNO) [81]. The latter further metabolizes to form NO [81], and/or H_2S displacing NO from *S*-nitrosothiols to liberate free NO [82]. Thus, it is postulated that impaired production of H_2S in HHcy may reduce some of the physiological aspects of NO-signaling. In fact, in a recent finding King et al reported that mice lacking CSE exhibited elevated oxidative stress, diminished NO levels due to dysfunctional eNOS in ischemia/reperfusion (I/R), and exacerbated myocardial injury [83]. These changes were ameliorated following H_2S therapy, suggesting H_2S-mediated cytoprotective signaling in I/R myocardium is dependent on eNOS activation and NO generation [83]. Using a similar model, Bos et al reported that CSE deficiency increased renal damage and mortality after I/R injury, whereas treatment with exogenous H_2S rescued CSE knockout mice from these injuries [84]. Further, overexpression of CSE in in vitro human embryonic kidney 293 cells (HEK293) was found to reduce ROS production [84]. Taken together, the findings above underline the role of endogenous H_2S in oxidative stress, and give direction to the mechanisms of H_2S related antioxidant effects.

On the other hand, although the role of reduced H_2S in CBS-deficiency remains largely unknown, H_2S activity is reported to occur partly through sulfhydration [85], a mechanism where NO acts through S-nitrosylation [86]. The literature on the effects of sulfhydration is however limited. It is therefore premature to generalize whether H_2S activity occurs through this mechanism. Nonetheless, this process is highly interesting as it could have a major role in physiology, but due to the lack of advanced techniques for accurately measuring sulfhydration it is not certain whether this process occurs or not. Further to H_2S generation and mechanism of vascular protection, Bearden et al reported an interesting finding that H_2S generating enzyme CBS and CSE are secreted into the bloodstream and generates H_2S through extracellular transsulfuration pathway, which protects endothelium from redox

stress [87]. Whether these mechanisms are universal or kidney vasculature has additional pathways to handle HHcy-stress is unknown and remains to be elucidated.

6.3. Homocysteinylation and protein malfunction

The consequence of high Hcy level has been studied in numerous in vivo and in vitro experimental models. The sulfhydryl group of Hcy forms stable disulfide bonds with cysteine residues in proteins altering their structure and function [62]. Recently, Hubmacher et al demonstrated that homocysteinylation affected functional properties of fibrillin and tropoelastin [88]. Fibrillin and tropoelastin are essential components for the formation of elastic fibers. Fibrillin contains intra-domain disulfide bonds which maintain its structural integrity and functional properties [89]. It is secreted into the extracellular matrix and becomes incorporated into the microfibrils to provide a scaffold for deposition of tropoelastin that is found in the elastic fibers of connective tissues including blood vessels. The N- and C-terminal regions of fibrillin colocalizes to form typical microfibril structure [90], and in this regard disulfide bond-mediated multimerization of the fibrillin-1 C-terminus and N- to C-terminal self-interaction are considered the initial steps for tissue microfibrils formation [91; 92]. Homocysteinylation reduces N- to –C-terminal fibrillin-1 self-interaction properties which is essential for biogenesis of microfibrils [88]. In addition, homocysteinylation on the disulfide bond in tropoelastin changes the self-assembly process which reduces elastic properties of blood vessels [88].

Although the protein modification by Hcy has focused on homocysteinylation, a metabolite of Hcy known as Hcy-thiolactone has the ability to form isopeptide bonds with protein lysine residues leading to a product termed as *N*-homocysteinylated protein (N-Hcy-protein) [93]. N-Hcy-protein has prothrombotic properties [94], and N-homocysteinylation triggers development of autoimmunity [95]. It is reported that serum levels of anti-*N*-homocysteinylated antibodies are positively correlated with the levels of plasma Hcy in CBS-deficient and stroke patients compared to their respective control and healthy subjects [34; 35; 96]. Thus protein homocysteinylation and autoimmune response may explain some of the unanswered pathologies found in renal disease patients, even though Hcy levels are at subclinical levels [96]. We and others have shown that H_2S supplementation offers renal protection in HHcy, especially by mitigating inflammation and ECM accumulation; however, the subtle mechanism is still incompletely understood. Because H_2S has a sulfur molecule, it is possible that H_2S may uncouple protein-S-S-Hcy bridge [97] resulting in dehomocysteinylation of protein. Another plausible mechanism is that H_2S may prevent the formation of *N*-homocysteinylated proteins thereby preventing protein modification and malfunction. These possibilities need to be explored in future.

6.4. Smooth muscle cell proliferation and alteration of vascular elastic compliance

The media layer in blood vessels comprise of vascular smooth muscle cells (VSMC) that serve as contractile component to regulate blood pressure. Any morphological or physiological dysregulation in these cells interfere with the vascular elastic compliance leading to alteration of blood pressure. Perry et al reported that H_2S inhibits airway smooth muscle cell proliferation through ERK-1/2 and p38 kinase pathway [98]. In an in vitro experimental model Zavaczki et al reported that H_2S inhibited phosphate induced

osteoblastic transformation and mineralization of VSMC [99]. In patients with chronic kidney disease undergoing hemodialysis, low plasma levels of H_2S was associated with decreased CSE enzyme activity. Although the Hcy levels in these patients were not measured, previous clinical studies have reported increased Hcy levels in hemodialysis patients [100; 101; 102]. Decreased CSE activity and low H_2S together with calcification and osteoblastic differentiation of VSMC suggests that Hcy may be involved in this process. Further studies are needed to dissect this mechanism.

6.5. Inflammation and autophagy

Reactive oxygen species (ROS) in pathological redox environment mediates tissue injury that facilitates inflammation through activation of pro-inflammatory molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[18; 19; 103]. While circulating leukocytes adhere to these molecules as a result of host defense mechanism [104; 105], transmigration of leukocytes to the sub-endothelial space depends on cytokines and chemokines which are released from the injured tissue [106]. In chronic inflammatory disorders, sustained elevation of ICAM-1 and VCAM-1 lead to aggregation of macrophages resulting in formation of plaques and atherosclerosis [107]. HHcy is a recognized pathophysiological stimulus of endothelial injury causing increased expression of ICAM-1 and VCAM-1 [108; 109]. In an experimental kidney model, we have shown increased macrophage infiltration, and expression of ICAM-1 and VCAM-1 associated with HHcy [19]. This inflammatory process was partially mitigated by H_2S treatment [19]. Since HHcy induced super oxide production and H_2S treatment diminished the oxidants in HHcy, it suggests that renal protection was secondary to the anti-oxidant properties of H_2S [18; 19].

ROS, including super oxide and hydrogen peroxide are important signaling molecules in normal and pathophysiological processes [110]. One of the age-regulating genes p66shc has recently been shown to exploit intracellular ROS levels by activating membrane-bound NADPH-oxidases, down-regulating antioxidant enzymes synthesis and mitochondrial ROS generation for cellular apoptosis [110]. Knockout mice of p66shc gene and various p66shc-deficient cell lines have exhibited higher resistance to agonist-induced oxidative stress and indicate the importance of p66shc in the stress-mediated apoptotic pathway [111; 112]. In addition, mice lacking p66shc showed increased resistance to ROS-dependent endothelial dysfunction and vascular inflammation compared to their wild type littermates [113; 114]. In a recent study, Kim et al reported that Hcy promotes endothelial dysfunction via p66shc transcription and hypomethylation of specific CpG dinucleotides in the p66shc promoter region [115]. In addition, the same group reported that knockdown of p66shc mitigated Hcy-induced adhesion of monocytes to EC indicating epigenetic mechanism of endothelial dysfunction and inflammation [115]. Whether H_2S has any regulatory role in Hcy-induced epigenetic mechanism of inflammatory processes and cellular dysfunction is yet to be investigated.

Oxidative damage of cellular organelles, particularly mitochondria may lead to mitochondrial autophagy or mitophagy [116]. Mitophagy is a natural physiological defense mechanism where damaged mitochondria through a mechanism of selective sequestration

and subsequent degradation are removed from the cells and the components recycled [117]. Mitochondria are the major site of oxidative phosphorylation for the production of ATP by oxidizing glucose, pyruvate and NADH [118]. This process of cellular respiration is dependent on oxygen and is known as aerobic respiration. Interestingly, ROS production is a part of aerobic respiration however, excess ROS can be detrimental to the cells [119]. In an earlier study, we demonstrated that HHcy initiated mitophagy through ROS generation and H_2S supplementation or delivery of H_2S producing gene, CBS, CSE and 3MST mitigated induction of mitophagy markers [43]. The beneficial effects of H_2S in Hcy-mediated mitophagy may occur by two different mechanisms: a) protection of mitochondria by scavenging ROS, and/or b) the triple gene (CBS, CSE and 3MST) overexpression metabolizing excess Hcy to produce H_2S thus preventing Hcy toxicity [43]. Further studies are needed to guarantee the safety and efficacy of gene delivery method for treating HHcy in pathological renovascular diseases.

6.6. Activation of matrix metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which cleave a wide variety of substrates including cell surface receptors, adhesion molecules, growth factors, cytokines, in addition to their well-recognized role in matrix components synthesis and degradation [120]. The expression and localization of MMPs in the kidney has been described in a recent review [121]. Resident and latent MMPs are activated by several mechanisms in a variety of renal pathologies including diabetic nephropathy, glomerular-tubulointerstitial fibrosis, inherited kidney disease, acute kidney injury and inflammation [18; 122]. The consequence of aberrantly activated MMPs in physiology are not only restricted to maintain ECM homeostasis, i.e. matrix degradation and synthesis, but also involved in the regulation of a wide array of cellular behaviors, such as proliferation, migration, differentiation, tumor growth and metastasis, epithelial-mesenchymal transition, angiogenesis, and apoptosis [120; 122; 123]. MMPs are synthesized as inactive zymogens that have a conserved cysteine residue which interacts with the active site of zinc rendering the protease inactive [124]. The activity of MMPs depends on disruption of this “cysteine switch”. Proteolytic cleavage of this switch can be accomplished by trypsin, plasmin, other MMPs or even nitrosative and oxidative stress [122]. In particular, cysteine residues within the MMPs propeptide domain are highly sensitive to oxidant-redox status, and ROS oxidizes cysteine residues and activates latent MMPs to active forms [121; 125; 126]. Hcy is known to generate ROS, and thus activate MMPs [18; 127]. In addition, Hcy-generated ROS, especially super oxide (O_2^-) reacts with NO forming peroxynitrite ($ONOO^-$) that reacts with active sites of MMPs increasing their activity [128]. Through nitration of tyrosine residues within MMPs, Hcy-generated $ONOO^-$ can also activate MMPs. In a recent review Steed and Tyagi postulated a plausible mechanism of MMP activation by this mechanism [129].

Since ROS and RNS activate MMPs, antioxidants are natural inhibitors of MMP activation. In this regard, H_2S is an antioxidant and free radicle scavenger [130] and therefore the inhibitory mechanism of redox-mediated MMP activity by H_2S is largely attributed though its antioxidant properties. Previously, in uninephrectomized one kidney (1-K) CBS+/− model we demonstrated that induction of MMP-2 and -9 correlated with the increased Hcy levels and decreased H_2S levels compared with their 2-K littermates [18]. The induction of

MMPs was due to increased formation of oxygen radicles in the kidney. Interestingly, H_2S supplementation mitigated O_2^- production and diminished MMP-2 and -9 activities suggesting the mechanism of Hcy-redox-H_2S pathway of renal MMP regulation in HHcy [18; 19]. It is also possible that H_2S may quench Zn^{2+} of gelatinases resulting in their inhibition. This Zn^{2+} quenching mechanism of MMP-2 and -9 inhibitions by H_2S has recently been reported by Talei et al in a hamster model of lung remodeling [131].

6.7. Extracellular matrix accumulation, glomerulosclerosis and renal dysfunction

Proteinuria is described as loss of protein from glomerular vasculature into urinary space and is the hallmark of ESRD [132; 133; 134]. Protein loss occurs due to disruption of slit diaphragm which is the final barrier of glomerular filtration consisting of interdigitating podocyte foot process with the neighboring cells [135; 136]. Podocytes are highly differentiated glomerular epithelial cells located in the glomerulus [137; 138]. In recent years, NADPH-derived ROS production has been implicated as a key mechanism triggering podocyte injury [139; 140; 141]. ROS activates matrix metalloproteinase in the glomerulus [142], and activated MMPs degrade matrix components including collagen/elastin [143]. However, since the turnover of collagen is faster than elastin [144; 145; 146; 147; 148] in a given time frame, excess collagen renders the vessel stiffer as the disease progresses. In addition, collagen in oxidative redox environment oxidizes [149], and renal peroxidation products deposits in the interstitial space leading to vascular fibrosis including glomerulo-tubulointerstitial fibrosis [150]. The early stages of glomerulosclerosis may be asymptomatic but proteinuria and fluid retention in the body indicates disease progression towards ESRD. Patients in ESRD require dialysis or kidney transplantation since other treatments for glomerulosclerosis are limited such as immunosuppressive drugs that prevent proteinuria [151]. Anti-hypertensive drugs such as ACE inhibitors are currently in use to preserve kidney function [152]. However, these drugs may cause further damage to the kidney. Therefore, developing an effective strategy that prevents early progression of kidney diseases associated with glomerulosclerosis may offer better management of this disease, particularly kidney failure associated with diabetes and hypertension. Since elevated Hcy level is known to be strongly associated with diabetes and hypertension induced nephropathy by deposition of excess ECM proteins in the glomerular-tubulointerstitial space, faster metabolism of Hcy or counteracting its effect in the microenvironment could be an important therapeutic tool for prevention or even slowing down disease progression. Using CBS+/− animal model of HHcy we have shown that uninephrectomy (1-kidney model) further increased Hcy levels in these mice and were associated with lower plasma H_2S levels and sign of proteinuria than their 2-kidney littermates [18]. Blood pressure was significantly increased along with a decline in renal function [19]. In addition, increased ROS, particularly superoxide production and ratio of glutathione-to-oxidized glutathione (GSH/GSSG) were increased in CBS+/− uninephrectomized mice with increased expression and activity of MMP-2 and -9 [18; 19]. Furthermore, increased collagen deposition and expression of inflammatory molecules ICAM-1 and VCAM-1, caused macrophage infiltration to sites of injury resulting in glomerular remodeling and a decrease in the filtration rate [19]. H_2S supplementation in these animals normalized microarchitecture and improved renal function suggesting that H_2S as a potential therapeutic agent for countering the adverse effects of HHcy [18; 19].

6.8. Renal mechanism of hypertension

In chronic kidney disease, low levels of plasma H_2S is often associated with a concomitant increase in plasma Hcy levels [5; 153]. The cause and effect relationship of HHcy in renal disease can therefore adversely affect the final outcome. Because Hcy is a precursor of H_2S [154; 155], changes in the Hcy metabolism and therefore H_2S synthesis can have a significant impact on HHcy-induced pathology. However, till date the mechanism by which HHcy causes vascular dysfunction and the role of H_2S in renal protection is incompletely understood. Four enzymes, CBS, CSE, 3MST and CAT metabolize Hcy to produce H_2S [156]. In renal disease and HHcy, impairment of CBS, CSE and 3MST enzymes leads to deficient H_2S production [18; 19; 157; 158]. Available literature suggests that HHcy induces oxidative stress, endothelial injury and causes down regulation of eNOS resulting in decrease NO production [68; 159; 160]. Activation of MMPs in the Hcy-redox environment leads to imbalance in extracellular matrix synthesis and degradation [161; 162]. Excess or oxidized collagen deposition in the renal microvessels causes glomerulosclerosis and tubulointerstitial fibrosis resulting in kidney remodeling, renal dysfunction and hypertension [163; 164; 165; 166]. We have shown that exogenous H_2S offers renal protection and mitigates hypertension by reducing oxidative stress, inflammation, and collagen deposition the HHcy kidney [18; 19]. Additionally in ex vivo renal artery, single, double or triple gene delivery of CBS, CSE and 3MST enzymes, we have demonstrated that conversion of Hcy to H_2S improves vascular relaxation [31]. However, further studies are needed for deeper understanding of Hcy-mediated pro-fibrotic and pro-inflammatory effects in renal vasculature and beneficial effects of H_2S therapy in improving renovascular function in diseases associated with HHcy.

6.9. Aerobic vs. anaerobic vascular relaxation: Roles of NO, CO, H_2S and Hcy

NO, CO and H_2S are a group of endogenously synthesized gasotransmitters having distinct physiological actions in the human body. Of the three, NO has been extensively studied. Oxygen is an important co-substrate for the generation of both NO and CO. Three isoforms of nitric oxide synthase (NOS), neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) have been recognized to catalyze the formation of NO from L-arginine and all three forms are expressed by the kidney. The activity of NOS requires stimulation by calcium-calmodulin but the dependence of calcium for nNOS and eNOS is higher than that of iNOS [167]. NO thus formed activates guanylate cyclase (GC) to increase the concentration of cyclic guanosine monophosphate (cGMP) which in turn activates cGMP-dependent protein kinase causing dephosphorylation of myosin light chain phosphatase ultimately leading to vessel relaxation [168; 169]. In the vascular smooth muscle cells, the upregulation and fate of cGMP is controlled by phosphodiesterase 5 (PDE 5) [167] [170]. Within the kidney, iNOS is produced in the proximal tubules and medulla under conditions of inflammation or sepsis and can result in oxidant injury [171]. The eNOS is expressed in the arterioles and glomerular capillaries and is involved in regulation of vascular tone.

CO is a product formed during heme degradation by heme oxygenase (HO) system which is mainly distributed in the spleen, liver and kidney. The isoform HO-1 is induced under conditions of oxidative stress, inflammation and sepsis; whereas, HO-2 and HO-3 are constitutively expressed [172]. The formation of CO-guanylate cyclase complex stimulates

the production of cGMP leading to cGMP-dependent signal transduction resulting in vasorelaxation [173]. Similar to NO, CO is involved in other actions such as neurotransmission, anti-proliferation of smooth muscle cells and inhibition of platelet aggregation. When compared to NO, CO is a weak vasodilator but under conditions of pathological stress where NO production is impaired, induction of HO-1 and the subsequent contribution of CO may play a significant role [174]. Support for CO induced vasodilation independent of NO production has been shown by the use of NOS inhibitor, Nω-nitro-L-arginine-methylester, (L-NAME) which demonstrated increased levels of cGMP [175]. In contrast, treatment with Tin protoporphyrin, SnPPIX, an inhibitor of HO showed decreased vasorelaxation induced by acetylcholine [176]. Taken together, the above studies suggest CO- cGMP signaling as an important mechanism involved in regulation of vascular tone. Upregulation of HO-1 has been shown to protect the kidneys from ischemia-reperfusion injury during renal transplantation [177]. Further, pre-treatment with molecules that release CO has been shown to increase the graft survival following kidney transplant and this effect has been attributed to its anti-apoptotic and anti-inflammatory activity mediated by cGMP pathway [178; 179].

Unlike NO and CO, the generation of H_2S by CBS/CSE/MST enzymes does not require oxygen as a substrate. The mechanism by which H_2S induces vasorelaxation involves activation of ATP-sensitive potassium channels (K_{ATP})[180; 181]. In a previous study involving arterio-venous fistula induced cardiac failure, we demonstrated that pretreatment with sodium thiosulfate reduced adverse extra-cellular matrix remodeling and improved cardiac function [182]. In the same study, an increase in H_2S production was associated with ventricular relaxation; this effect could be due to hyperpolarization of K_{ATP} channels inhibiting Ca^{++} entry into the cells. A second possible mechanism may involve the chelating property of sodium thiosulfate causing a reduction in free Ca^{++} intracellular compartments [182]. A significant down regulation of H_2S producing enzymes has been observed in chronic kidney disease suggesting that H_2S deficiency may contribute to the disease process [157]. Indeed, in an earlier study we found that kidneys from diabetic mice had decreased H_2S levels and underwent adverse renal remodeling involving N-methyl-D-aspartate receptor 1 (NMDAR1) mediated upregulation of connexin-40 and -43 [183]. H_2S treatment has been reported to reduce renal injury and improve renal function following ischemia-reperfusion injury in murine models [184; 185]. The beneficial effects of H_2S treatment is possibly due to its multiple roles in modulating vascular tone, inflammation and oxidative stress.

Interestingly in a recent report, Szabo et al found that both H_2S and NO are interdependent in mediating vasorelaxation and development of new vessels [186]. In addition, vasorelaxation appears to be secondary to a reduction in PDE5 activity thereby decreasing breakdown of cGMP [186; 187]. H_2S is also known to increase NO synthesis by upregulation of eNOS [188]. Together, these studies indicate significant cross-talk between gasotransmitters and appear to maintain reciprocal regulation in various vascular functions. Possible pathways of aerobic vs. anaerobic vascular relaxation and crosstalk between NO, CO and H_2S is depicted in Figure 3.

7. Concluding remarks and perspectives

Hcy is a methionine metabolite non-protein amino acid which is the homologue of cysteine, differing by additional methylene bridge (-CH_2-). Although kidney is a major site for Hcy metabolism, a small portion of Hcy is also excreted by the kidney. However, in chronic kidney diseases, as renal function declines, plasma levels of Hcy increases due to volume retention. This in turn, further contributes to renovascular injury through diverse mechanisms which includes oxidative stress, protein homocysteinylation, inflammation, autophagy, activation of latent MMPs, ECM deposition and alteration of renal-vascular elastic properties resulting in exacerbated kidney dysfunction. Renal dysfunction, volume retention and Hcy accumulation is a vicious cycle causing renal mechanism of hypertension.

Interestingly, many of the adverse effects of Hcy are counteracted by the gaseous molecule H_2S, which is one of its natural metabolites. H_2S is produced by desulfuration of Hcy (or cysteine) in the transsulfuration pathway catabolized by CBS, CSE, 3MST and CAT enzymes. It functions as endogenous signaling molecule to regulate a wide array of physiological processes including cellular oxidative stress. Hcy is known to cause oxidative stress that activates MMPs. In pathological state, active MMPs lead to adverse remodeling causing an imbalance of collage/elastin ratio resulting in glomerular-tubulointerstitial fibrosis which progresses to renal dysfunction. Since H_2S is an antioxidant and vasodilator, treatment with H_2S reduces oxidative stress and normalizes vascular compliance in kidney diseases associated with HHcy. Although at our current understanding it is not clear whether H_2S dehomocysteinylates protein; it is apparent that the benefit of H_2S treatment in HHcy is in part by reduction of Hcy-induced oxidant stress irrespective of plasma Hcy levels. In addition, CBS, CSE and 3MST gene therapy can metabolize Hcy to produce H_2S and thus render better management of Hcy handling in pathological HHcy.

H_2S shares many of the physiological processes rendered by CO and NO, although few fundamental differences exist in terms of their generation and mechanisms of action. For example, unlike NO and CO, generation of H_2S does not require oxygen as substrate and therefore sustains mitochondrial ATP generation under hypoxic condition [189]. In a recent report, Modis et al proposed that intramitochondrial H_2S producing pathway may serve a physiological role to maintain mitochondrial electron transport and cellular bioenergetics [190]. We previously reported that H_2S regulated adenylate cyclase VI (AC_{VI}), an enzyme which converts ATP to cAMP in cardiac tissue [182]. It would be interesting to study whether the conversion of Hcy to bio-friendly H_2S in HHcy may lead to ATP generation and subsequent cAMP production, which may vasodilate arterioles to improve renal function under diseased states.

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Highlights

- Renal volume retention in chronic kidney disease increases plasma Hcy levels
- Hcy metabolism produces physiologically important hydrogen sulfide molecule
- H_2S production is impaired in chronic kidney disease
- H_2S deficiency is associated with vascular dysfunction and matrix remodeling
- H_2S treatment mitigates HHcy-associated renal dysfunction

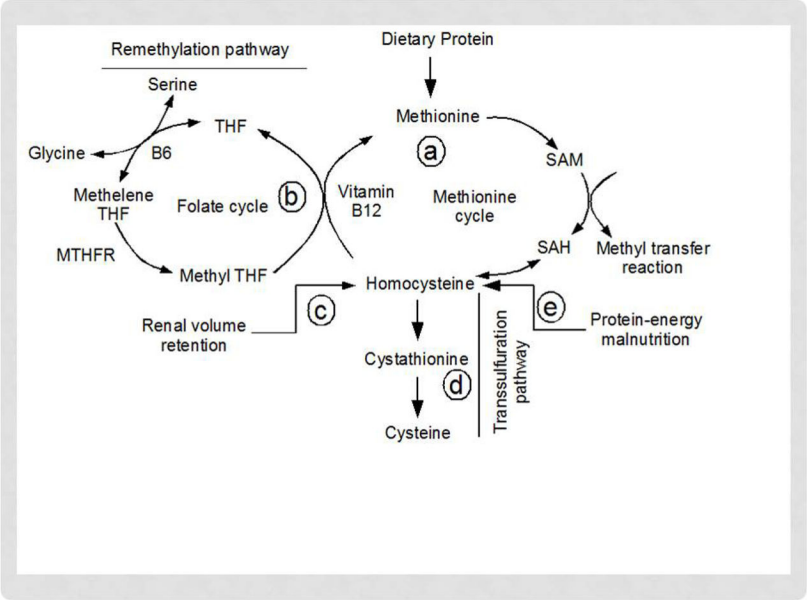

Figure 1.

Homocysteine synthesis, accumulation and metabolism pathways. MTHFR, Methelene tetrahydrofolate reductase; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; THF, tetrahydrofolate reductase.

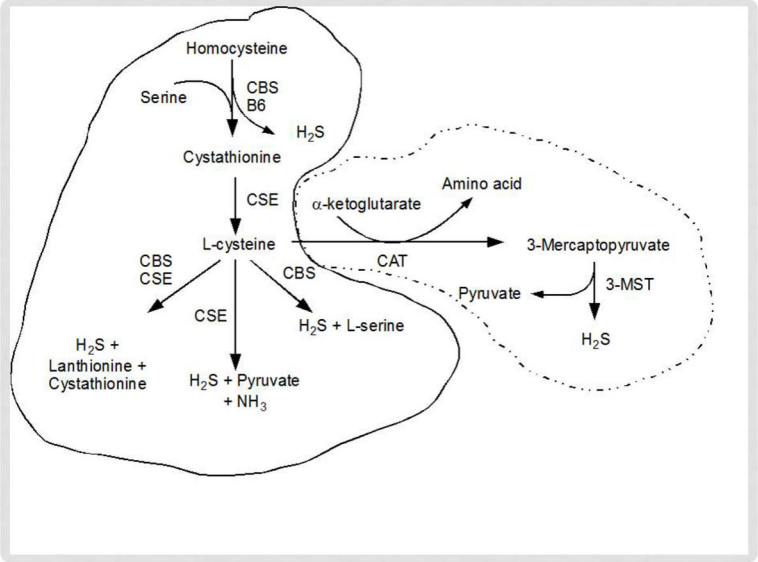

Figure 2.

Pathways for hydrogen sulfide (H2S) production from homocysteine (Hcy). Cytosolic enzymes, CBS and CSE metabolizes Hcy to H2S (lined enclosure); whereas cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3MST) metabolizes Hcy to H2S in both cytosol and mitochondria (dotted enclosure).

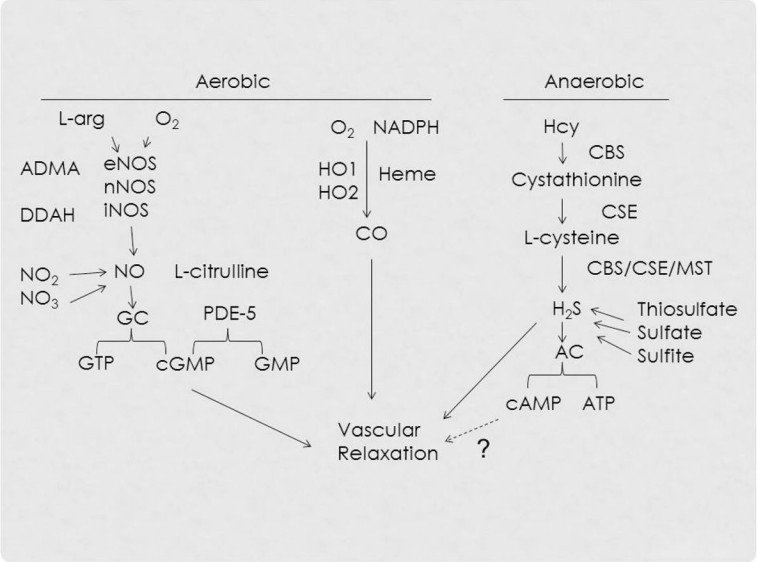

Figure 3.

H_2S mechanism of anaerobic vs. NO and CO mechanisms of aerobic vascular relaxation. ADMA, asymmetric dimethyl arginine; ATP, Adenosine triphosphate; cGMP, Cyclic guanosine monophosphate; cAMP, Cyclic adenosine monophosphate; DDAH, dimethylarginine dimethylaminohydrolase; PDE-5, phosphodiesterase type 5; GC, guanylate cyclase; GTP, guanosine triphosphate; NADPH, Nicotinamide adenine dinucleotide phosphate