A CRITICAL REVIEW ON PHARMACOLOGICAL SIGNIFICANCE OF HYDROGEN SULFIDE (H$_2$S) ON NF-κB CONCENTRATION AND ICAM-1 EXPRESSION IN RENAL ISCHEMIA REPERFUSION INJURY

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Abstract: Until recently hydrogen sulfide (H$_2$S) was the least appreciated of the three gasotransmitters but now recognized as 3rd gaseous mediator after nitric oxide (NO) and carbon monoxide (CO). H$_2$S regulates a number of physiological processes like vasorelaxation, prevention of inflammation, leukocyte adhesion, anti-proliferative effects, anti-thrombotic effects, resistance to oxidative stress and protection against ischemia reperfusion injury (IRI). However, considerable amount of research is still needed to evaluate the mechanisms involved in the therapeutic effects of H$_2$S in IRI such as its effects on nuclear factor-kappa B (NF-κB) concentration and intercellular adhesion molecule-1 (ICAM-1) expression in renal IRI and ARF (acute renal failure). More than a decade of good repute among researchers, H$_2$S research has certain results that need to be clarified more such as whether H$_2$S is pro-inflammatory or anti-inflammatory agent. Moreover, pathways adopted by H$_2$S in the protein modification and its effects on cell signalling specially its effect on NF-κB in the process of inflammation are not fully elucidated. H$_2$S has delighted researchers and a great deal of information is being generated every year. The main purpose of the review is to provide an update on the development in the research of H$_2$S in renal IRI due to uncertainty of the exact role of H$_2$S on ICAM-1 expression and NF-κB concentration whether it inhibits or activates them.

Keywords: hydrogen sulfide, renal ischemia reperfusion injury, NF-κB, ICAM-1

History and background of H$_2$S

H$_2$S is a colorless gas with the characteristic foul odor of rotten eggs; it is heavier than air, very poisonous, corrosive, flammable and explosive. H$_2$S is known as a toxic gas with rotten egg smell for more than 300 years. As a toxicant, H$_2$S mainly damages the brain, kidneys, and lungs (1). Some studies have also reported the toxic effects of H$_2$S on the central nervous system (CNS) and respiratory system (2, 3). The human body produces small amount of H$_2$S and uses it as a signalling molecule (4).

H$_2$S is now a recognized gaseous mediator and induces many and varied biological effects (5). Since the last decade, H$_2$S has acquired the attention of researchers because of its significant role in different systems of the body. Numerous therapeutic potentials of H$_2$S has been explored including vasorelaxation (6-10), anti-hypertensive effects (11), anti-proliferative effects (12), anti-thrombotic effects (13), prevention of inflammation and leukocyte adhesion (14), resistance to oxidative stress (15, 16) and protection against IRI (17).

H$_2$S production, storage and metabolism

H$_2$S is produced endogenously via the metabolism of cysteine and/or homocysteine (18, 19) by the enzymes cystathionine-β-synthase (CBS) (19, 20) and cystathionine-γ-lyase (CSE) (19, 21). Both enzymes CSE and CBS are present in mammalian tissues. 3-mercaptopyruvate sulfur transferase (3-MST) can also generate H$_2$S along with cysteine aminotransferase (CAT) to metabolize cysteine generating pyruvate and H$_2$S (19, 22). CBS is a major contributor of H$_2$S production in the brain whilst CSE levels predominate in most peripheral tissues. 3-MST appears to contribute to H$_2$S production in both the peripheries and CNS (22, 23). In the vascular system CSE is primarily expressed in vascular smooth muscle cells but evidences are also available that it is also expressed in the endothelium (24, 25). H$_2$S is metabolized by mitochondrial oxidative mod-

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Sulfide metabolism (26). H₂S consumption in the presence of O₂ is high (27) thus, H₂S production is offset by rapid clearance resulting in low basal levels of H₂S. In addition of high clearance H₂S may also be stored as acid-labile sulfur (28) or bound sulfane sulfur with in cells (29).

**Physiological concentration of H₂S**

The endogenous concentration of circulating H₂S is 50–160 μM in rats, bovines, and in humans (30, 31). Various reports are available in the literature that has identified different physiological concentrations of H₂S in the laboratory rats. The endogenous concentration of H₂S in the rat serum has been reported to be approximately 46 μM (32). The results of one research group showed that in WKY rats the normal concentration of H₂S is 10 μM (33) however, another published data has reported that the plasma level of H₂S is 50 μM in experimental rats (6). The tissue level of H₂S has been thought to be higher than the plasma level (19).

Endogenous concentration of H₂S in the brain has been reported in the range of 50–160 μM (34). At physiological concentration H₂S hyperpolarizes the membranes of localized cells, modulates the neuronal excitability, relaxes the smooth muscles and controls cell apoptosis or proliferation (6, 30, 31, 35, 36). Significant changes have been observed in the concentration of H₂S because of various diseases. The level of endogenous H₂S concentration decreased below the normal level in coronary heart disease (37) of spontaneously hypertensive rats (38). However, endogenous concentration of H₂S increased in carrageenan-induced inflammation model (39), acute pancreatitis (40), hemorrhagic shock (41), and in endotoxin shock (42, 43). The vasorelaxant effects of H₂S has been proven (6) which shows that H₂S relaxes the isolated aorta at a concentration as low as 18 μM and 60 μM. It has been reported that the generation of endogenous H₂S by CBS and CSE limits renal ischemia reperfusion injury (IRI). In recent years, H₂S has emerged as an important biologically active compound due to its physiological role in health and disease states. Along with its beneficial effects some unwanted effects of exogenous H₂S donors are also available in the literature. Administration of H₂S donor (14 μM/kg) resulted in lung inflammation and increased lung and liver myeloperoxidase (MPO) activity and plasma tumor necrosis factor-α (TNF-α) concentration (43). Exogenously, H₂S (180 μM/kg) significantly increases the infarct size after permanent occlusion of the middle cerebral artery (44).

**H₂S as a reactive oxygen species scavenger**

Reactive oxygen species (ROS) can be divided into free radicals such as superoxide(O₂⁻·) and hydroxyl (OH⁻) radicals and non-radicals such as hydrogen peroxide (H₂O₂) and reactive nitrogen species such as peroxynitrite (ONOO⁻). There are multiple sources for the genesis of ROS including mitochondria, cyclooxygenase, xanthine oxidase and NADPH oxidase (45). In mammalian tissues ROS such as superoxide (O₂⁻·) is produced under both pathological and physiological conditions. They are essential for the immunological defence mechanism of phagocytes however, overproduction of ROS and the resulting oxidative stress have been reported to be involved in different types of diseases (46). Elevated levels of ROS can compromise the antioxidant defence mechanism of the cells and react with cellular macromolecules such as lipids, proteins, membrane bound polyunsaturated fatty acids and DNA leading to irreversible cellular damage (47). Superoxide is the parent ROS molecule in all cells. It can be generated by NADPH oxidase, uncoupled endothelial nitric oxide synthase (eNOS), the mitochondrial enzyme complex cytochrome P450 and xanthine oxidase (45). H₂S is a potent one-electron chemical reductant and nucleophile that is theoretically capable of scavenging free radicals by single electron or hydrogen atom transfer. Thus, H₂S may participate in many reactions (48) and is reported to readily scavenger reactive oxygen and nitrogen species such as peroxynitrite (49) superoxide (50), hydrogen peroxide (51) and hypochlorous acid (52). However, the kinetics reactivity and mechanism of H₂S /HS⁻ interactions with ROS is poorly understood under physiological conditions. H₂S has been reported to inhibit superoxide production in human endothelial cells (53) and vascular smooth muscle cells (54) by reducing nitric oxide xanthine (NOX) oxidase expression and activity. However, it is not known if this activity is physiologically relevant or whether H₂S can protect against oxidative stress driven vascular dysfunction. In addition, H₂S is reported to increase glutathione levels and bolster endogenous anti-oxidant defence system (55).

Collectively, these findings suggest that this molecule have anti-oxidant potential but its exact mechanism of action is not clear.

**H₂S as an inhibitor of reactive oxygen species formation**

H₂S has also been shown to be important in regulating mitochondrial function (56) and can reduce mitochondrial ROS formation (57).
Hyperglycaemia induced overproduction of ROS was reversed with H2S treatment and furthermore, we were informed that endogenously produced H2S acts to protect endothelial function from hyperglycaemic oxidative stress (58). NaHS (H2S donor) induced protective effects were synergistic with endogenous anti-oxidants (50). This suggests that H2S is capable of reducing the production of H2O2, ONOO- and O2 in a time and concentration dependent manner. The mechanism of this effect was not established however H2S at nanomolar concentrations has been reported to inhibit superoxide production in human endothelial cells (53).

**Effect of H2S on renal ischemia reperfusion injury**

Ischemia reperfusion injury (IRI) is one of the common causes for delayed function of renal allograft and is associated with poor long-term renal function. Complete and prolonged interruption of renal arterial blood flow occurs during renal transplantation or surgical procedures such as nephrolithotomy, parenchymal sparing surgery for renal tumours, and renal arterial surgeries. Prolonged renal ischemia can lead to acute renal failure (ARF) so, it exerts more pressure over healthcare setups due to limited availability of kidney donors (59). It has been estimated that ischemic insult especially during renal transplantation is responsible for 20-30% primary graft dysfunction (60). It has been demonstrated that homocysteine (Hcy) plays a detrimental role in ischemia reperfusion in the kidneys (61). The generation of endogenous H2S may either limit or contribute to the degree of tissue injury caused by IRI. In order to investigate this approach a study was conducted (17). The findings of this study suggest that (i) the synthesis of endogenous H2S by CSE is essential to protect the kidney against ischemia reperfusion injury and dysfunction and aids in the recovery of renal function following ischemia reperfusion; (ii) H2S generated by NaHS reduced IRI and improved renal dysfunctions and (iii) the observed protective effects of H2S are due to both anti-inflammatory and anti-apoptotic effects. So the mentioned study suggested that restoration of CBS-mediated H2S synthesis may exert a renal protective effect against IRI.

**Effects of H2S on ICAM-1 expression in renal ischemia reperfusion injury**

ICAM-1 (intercellular adhesion molecule-1) potentiates neutrophils & endothelial interaction (63). Neutrophils infiltration are important mediators of IRI (64). Mononuclear cell infiltrates are found in human renal IRI particularly T-lymphocytes (65). Leukocyte integrins are one of the mediators of the renal IRI (66). Lymphocytes require engagement with adhesion molecules to extravasate into the parenchyma of the injured kidney. Upregulation of ICAM-1 has been found in post ischemic human & animal kidneys (63) and blockade of ICAM-1 has attenuated the renal IRI in the animal models (67-70). Leukocyte adhesion molecules potentiate neutrophils recruitment in IRI and the concentration of these neutrophils increases further more in IRI. Neutrophils recruited during reperfusion have been reported as mediators of renal parenchymal injury in ischemic ARF (66). Oxygen metabolites have been shown to increase expression of ICAM-1 which in response increases neutrophils rolling, adhesion, infiltration and its retention which induces an inflammatory response and thus increases the extent of injury (71). Leukocyte expresses the adhesion molecules CD11/CD18 on their surface. Thus CD11/CD18 leukocyte adhesion pathways plays a role in mediating ischemic ARF in rats (72). Leukocytes adhesion molecule increases neutrophils recruitment which in response increases reperfusion injury. Thus the blockade of CD11/CD18 integrins & ICAM-1 attenuates ARF of renal ischemia (73). The ARF promotes neutrophils adherence via CD11a/CD18b-dependent interaction with ICAM-1 that appears to be mediated by hydrogen peroxide & platelet-activating factor (PAF).

Studying the role of H2S in renal IRI we were informed by a reported study that endogenously produced H2S has anti-inflammatory properties which has been attributed to the inhibition of leukocytes adhesion, rolling and its transmigration to the reperfused kidney (74). The findings of this research contradicted the results of another documented study which have demonstrated that endogenously produced H2S is so low to produce inhibitory effects on leukocyte adhesion, rolling and its transmigration (75). So, according to this study, endogenously produced H2S by CSE is a pro-inflammatory mediator by increasing the leukocyte adhesion, rolling and transmigration. Another finding cropped up supporting the claim that H2S
donor (NaHS) reduces IRI by attenuating the oxidative stress (76). So, the results of this study suggested that NaHS as well as other H₄S endogenous donors modulate leukocyte-mediated inflammation by decreasing leukocyte adhesion and leukocyte infiltration through activation of K ATP channel.

**Effect of H₄S on NF-κB in renal ischemia reperfusion injury**

ROS stimulates nuclear factor kappa-B (NF-κB) (77) which in response stimulates the expression of ICAM-1 and ICAM-1 induces leukocytes adhesion, rolling and then its infiltration to the targeted area where it induces the inflammatory process. In non-stimulated cells NF-κB resides in the cytoplasm in an inactive complex with the inhibitor IκB (inhibitory kappa-B) protein (77, 78). Various cellular damaging stimuli like ROS causes the degradation and release of IκB from NF-κB which then allows NF-κB to enter into the nucleus where it binds with DNA and controls its transcription thereby inducing the synthesis of mRNA (77). Various stimuli activates IκB kinase complex which then phosphorylates IκB protein (78) and causes the degradation of IκB which in response causes the activation of NF-κB and its translocation to the nucleus and the expression of its various genes. The effects of H₄S to inhibit the activation of NF-κB and to reduce the expression of ICAM-1 in renal IRI was confirmed by a research group (17). The results of this study showed that NaHS inhibited the activation of NF-κB and reduced the expression of ICAM-1. So, these findings suggest that the synthesis of endogenous H₄S is essential to protect the kidney against IRI and renal dysfunction by providing beneficial effects which aid in the recovery of renal function following IRI. Another school of thought have also reported that H₄S reduced pro-inflammatory cytokines expression by inhibiting NF-κB and reduced leukocyte endothelial adherence by reducing adhesion molecule expression (79).

**Future prospects**

In view of the above mentioned literature review many things remained to be explored and many questions still to be answered. One of the major ambiguities is the role of H₄S in inflammation process. Future studies must clarify more whether H₄S is pro-inflammatory or anti-inflammatory mediator in renal IRI and ARF. The molecular mechanism involved in the inhibition of NF-κB regarding the oxidative stress modification by H₄S should be investigated because so far its mechanism is not clearly identified. Endogenous concentration of H₄S is decreased in renal IRI and ARF. Two different schools of thought have reported that impaired activity of CBS and CSE alone can decrease endogenous production of H₄S. So, this should be furthermore investigated which one of the two enzymes is the most important one regarding renal IRI.

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