

# Protective effect of hydrogen sulfide on the kidney (Review)

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Received December 25, 2020; Accepted June 22, 2021

DOI: 10.3892/mmr.2021.12335

**Abstract.** Hydrogen sulfide (H<sub>2</sub>S) is a physiologically important gas transmitter that serves various biological functions in the body, in a manner similar to that of carbon monoxide and nitric oxide. Cystathionine-β-synthase, cystathionine-γ-lyase and cysteine transaminase/3-mercaptopyruvate sulphotransferase are important enzymes involved H<sub>2</sub>S production *in vivo*, and the mitochondria are the primary sites of metabolism. It has been reported that H<sub>2</sub>S serves an important physiological role in the kidney. Under disease conditions, such as ischemia-reperfusion injury, drug nephrotoxicity and diabetic nephropathy, H<sub>2</sub>S serves an important role in both the occurrence and development of the disease. The present review aimed to summarize the production, metabolism and physiological functions of H<sub>2</sub>S, and the progress in research with regards to its role in renal injury and renal fibrosis in recent years.

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**Key words:** hydrogen sulfide, ischemia-reperfusion injury, drug nephrotoxicity, diabetic nephropathy, renal fibrosis

## 1. Introduction

Hydrogen sulfide (H<sub>2</sub>S) was initially considered a toxic gas; however, with the continuation of research, it has been revealed to serve an important role in living organisms, becoming another important gas transmitter, alongside carbon monoxide (CO) and nitric oxide (NO) (1,2). Since H<sub>2</sub>S has been confirmed to be present in mammalian tissues, a large number of studies have suggested that H<sub>2</sub>S can exert anti-inflammatory, anti-oxidative stress and anti-fibrotic effects in the body (3,4). Previous studies have confirmed that H<sub>2</sub>S serves a physiological and pathological role in the cardiovascular system, brain and nervous system (5-7). However, due to the uneven distribution of H<sub>2</sub>S-generating enzymes in various organs and tissues, the concentration of H<sub>2</sub>S differs widely in different organs (8). The study of the underlying mechanisms of H<sub>2</sub>S in physiological and pathological processes in the kidney may assist in systematically understanding its molecular biological mechanisms, particularly with regards to its renoprotective role.

## 2. General physicochemical properties of H<sub>2</sub>S

H<sub>2</sub>S is a colorless gas that smells similar to rotten eggs; the smell of H<sub>2</sub>S can be picked up by the human olfactory system when the concentration in the air reaches 1/400 of its toxic level (9). As a weak acid, H<sub>2</sub>S dissociates in water to reach equilibrium at room temperature (25°C) with a pK<sub>a1</sub> of 6.97-7.06 and pK<sub>a2</sub> of 12.35-15.0. Moreover, H<sub>2</sub>S in aqueous solution is volatile, and its mutual conversion between the liquid phase and the gas phase reaches equilibrium, as shown in Fig. 1; this balance is affected by ambient temperature, pressure and other solutes in the aqueous solution (10). In addition, H<sub>2</sub>S is highly lipophilic, which not only allows it to have a higher concentration under fat-abundant conditions, but also allows it to freely penetrate lipid biofilms without relying on membrane channels to exert its biological activity (11). Since H<sub>2</sub>S and HS<sup>-</sup> coexist in solution, it is difficult to make a clear distinction between which of them has a role in biological mechanisms or whether they both have biological effects.

## 3. Generation and metabolism of H<sub>2</sub>S

**Generation of H<sub>2</sub>S.** The synthesis of H<sub>2</sub>S in mammals primarily relies on enzymatic pathways. Three traditional enzyme systems

that catalyze  $H_2S$  production include the synergistic action of cystathionine- $\beta$ -synthase (CBS), cystathionine- $\gamma$ -lyase (CSE) and cysteine transaminase (CAT) with 3-mercaptopyruvate (3-MP) sulphotransferase (3-MST) (12,13). With pyridoxal phosphate (also known as vitamin B6) as a cofactor, CSE and CBS are responsible for the majority of endogenous  $H_2S$  generated, as shown in Fig. 2. L-cysteine is catalyzed by CSE or CBS to produce  $H_2S$  and L-serine, or by CBS to produce pyruvate,  $NH_3$  and  $H_2S$ . CSE can polymerize two L-cysteine residues into L-cystine, and then CSE uses L-cystine as a substrate to decompose it into thiocysteine, pyruvate and  $NH_3$ . The generated thiocysteine reacts with other thiols to generate  $H_2S$  through a nonenzymatic reaction. In addition, L-cysteine polymerizes with L-homocysteine as substrates for CSE or CBS to produce L-cystathionine and  $H_2S$ . L-cystathionine is further decomposed by CSE into L-cysteine,  $\alpha$ -ketobutyrate and  $NH_3$ , and L-cysteine circulation is achieved (12,13). It has been reported that in the reaction in which L-cysteine is metabolized to  $H_2S$  via CBS, the amount of  $H_2S$  produced by  $\beta$ -replacement is 50X that of  $\beta$ -elimination (14). During the production of  $H_2S$  by CSE, the  $\alpha$ ,  $\beta$ -elimination of cysteine is the primary source of  $H_2S$ , accounting for 70% of  $H_2S$  production (15).

Unlike CSE and CBS, 3-MST uses metallic zinc as a cofactor (14). Moreover, L-cysteine must be converted into 3-MP and L-glutamic acid through the reaction of CAT with  $\alpha$ -ketoglutarate, and 3-MP is then desulfurized by 3-MST as a direct substrate to produce  $H_2S$  and pyruvate (16,17). In peroxisomes, D-amino acid oxidase catalyzes D-cysteine, instead of L-cysteine, to produce 3-MP,  $NH_3$  and  $H_2O_2$  in the presence of water and oxygen, and the resulting 3-MP is transferred to mitochondria for 3-MST utilization to generate  $H_2S$  (18). The entry of 3-MP in peroxidase into mitochondria is generally in the form of vesicles, as shown in Fig. 2. Clinical observations have reported that the synthesis of CSE and CBS in patients with chronic kidney disease is reduced, whereas the expression of 3-MST and hemorrhagic homocysteine is increased (19). This may be explained by the specific mechanism of action used by the aforementioned enzymes to generate  $H_2S$ . When the production of  $H_2S$  by CBS and CSE via the L-homocysteine/L-cystathionine pathway is reduced, the utilization of L-homocysteine is restricted, and the patient may present with hyperhomocysteinemia.

**Metabolism of  $H_2S$ .**  $H_2S$  in the body is primarily metabolized by mitochondria (20). Sulfoquinone oxidoreductase (SQOR) in the mitochondria can utilize  $H_2S$  and metabolize it into thiosulfate with the assistance of thiosulfate sulfur transferase (TST) and thiodioxygenase (ETHE1). During this process, reduced glutathione serves an important role, and thiosulfate is further oxidized under the action of thiosulfate reductase and sulfite oxidase (SUOX), and finally excreted in the form of sulfate through the kidneys, as shown in Fig. 3. The role of  $O_2$  in this process is irreplaceable (21,22). Notably, coenzyme Q (CoQ) is closely related to the aforementioned enzymes. A previous study revealed that the absence of CoQ may induce downregulation of the expression levels of thioquinone oxidoreductase, TST, ETHE1 and SUOX (23). During the early stages of CoQ deficiency, SQOR levels are significantly decreased, affecting  $H_2S$  oxidation, and CoQ supplementation can save  $H_2S$  metabolism without affecting its production (24). While SQOR

activity and protein levels decrease, protein levels of the other mitochondrial enzymes (TST, ETHE1 and SUOX) in the  $H_2S$  oxidation pathway increase in fibroblasts; however, it is not clear whether the increase in the levels of several enzymes is a temporary increase in compensation or inversely proportional to the decrease in SQOR levels (23). Therefore, it is important to explore the effect of CoQ deficiency on  $H_2S$  metabolic enzymes, which may assist in studying the regulation of  $H_2S$  concentration through  $H_2S$  metabolic pathways to affect several signaling pathways in the body.

Under normal physiological conditions, when  $H_2S$  production in tissues exceeds utilization metabolism, another metabolic pathway, cytoplasmic methyltransferase methylation, is required. To date, the known methyltransferases in the human body are thiopurine methyltransferase (TPMT) and thiol methyltransferase (TMT). TPMT selectively methylates thiopurine compounds, whereas TMT selectively methylates aliphatic mercaptan substrates. Using mass spectrometry to directly measure the formation of methyl sulfide, the methylation of  $H_2S$  and the obtained kinetic curves have previously been assessed; the  $K_m$  of methylation of  $H_2S$  was  $146.2 \pm 29.2 \mu\text{mol}$  (25). It has also been demonstrated that human methyltransferase-like protein 7B can catalyze the transfer of a methyl group from S-adenosine 1-methionine to  $H_2S$  and other exogenous mercaptan small molecules, thereby metabolizing  $H_2S$  (25). In addition,  $H_2S$  can be removed by methemoglobin or metallic/nonmetallic molecules, such as oxidized glutathione (26).

#### 4. Physiological role of $H_2S$ in the kidney

**Renal excretory function.** Clinical studies have confirmed that plasma  $H_2S$  levels are positively correlated with glomerular filtration rate in patients with chronic kidney disease (CKD). In addition, serum homocysteine content in patients with advanced CKD (CKD3-5) has been reported to be significantly higher than that in patients with early CKD (CKD1-2), and increases in serum homocysteine levels are associated with decreased renal function (19). Hyperhomocysteinemia has been shown to aggravate the deposition of extracellular matrix (ECM) proteins and the destruction of connexin, and lead to the phosphorylation of endothelial NO synthase (eNOS) in renal vascular endothelial cells, thereby reducing the bioavailability of NO to induce vasoconstriction and decrease renal blood flow, which is manifested by a decrease in plasma  $H_2S$  levels and glomerular filtration rate (GFR) (27).  $H_2S$  can increase urinary sodium and potassium excretion by inhibiting Na-K-2Cl co-transporters and Na-K-ATPase. *In vivo* experiments have shown that intra-renal artery infusion of the  $H_2S$  donor NaHS may increase renal blood flow, GFR and excretion of urinary sodium [ $U(\text{Na}) \times \text{volume}$ ] and potassium [ $U(\text{K}) \times \text{volume}$ ], and the infusion of L-cysteine via the renal artery to increase the concentration of  $H_2S$  substrate could simulate this effect (28). In addition,  $H_2S$  may block the opening of phosphatidylinositol 3,4,5-triphosphate-dependent distal renal epithelial sodium channels induced by  $H_2O_2$ , reduce the reabsorption of sodium by nephrons and increase urinary sodium excretion (29). In addition, the use of CSE and CBS enzyme inhibitors propargylglycine and amino-oxoacetate has been shown to increase urine volume and decrease urine osmotic pressure in mice; this is related to the  $H_2S$ -induced decrease in the

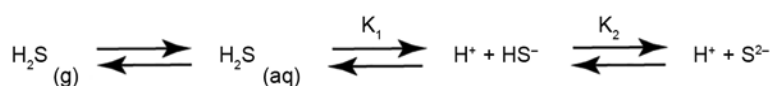


Figure 1. Dissociation equilibrium of H<sub>2</sub>S in aqueous solution (25°C). K<sub>1</sub> and K<sub>2</sub> represent the equilibrium constants when H<sub>2</sub>S and HS reach dissociation equilibrium in aqueous solution, respectively. H<sub>2</sub>S, hydrogen sulfide; g, gaseous; aq, aqueous.

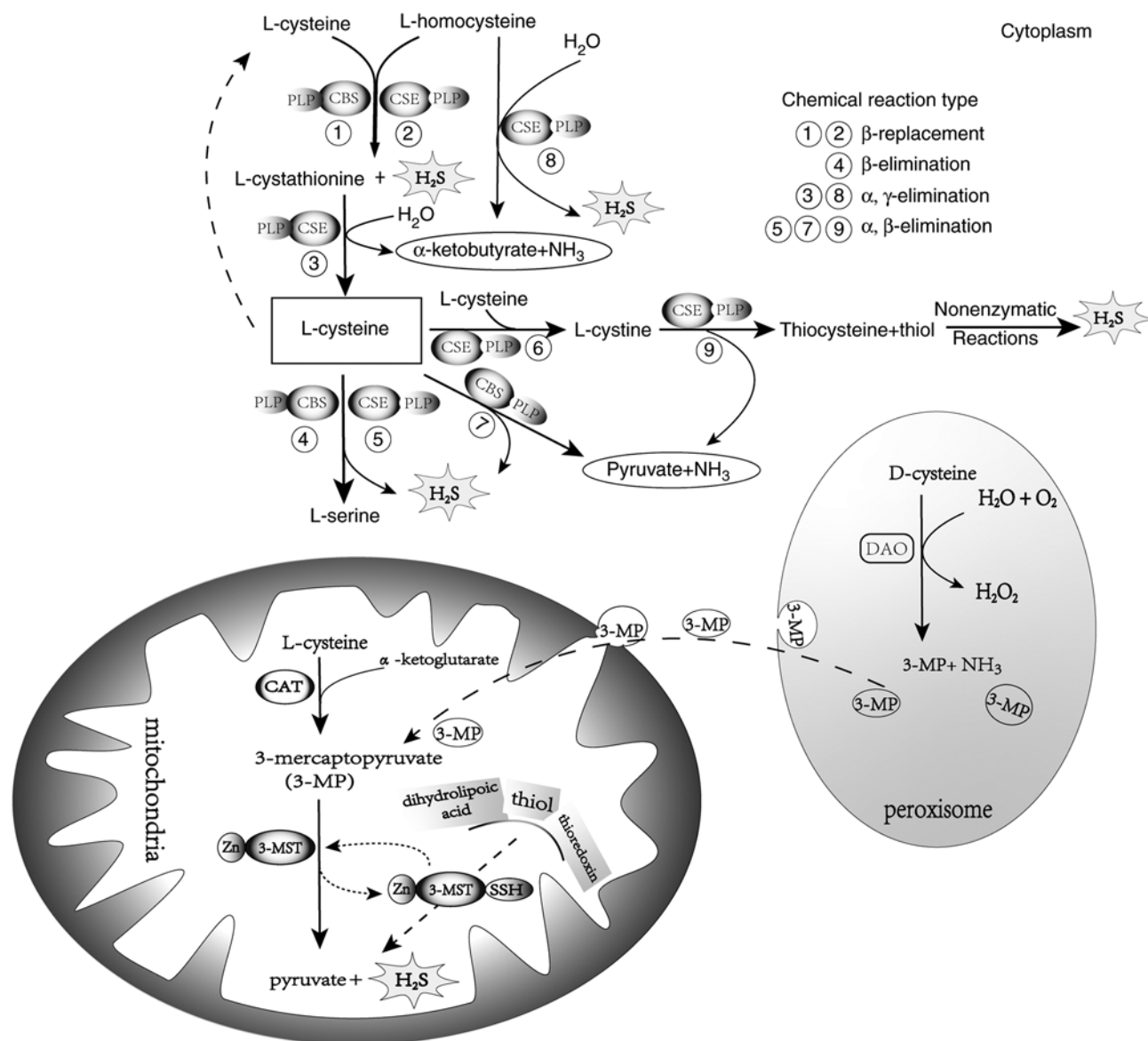


Figure 2. In the cytoplasm, 1-3: CSE or CBS catalyzes the β-replacement reaction of L-cysteine and L-homocysteine to polymerize and form L-cystathionine and H<sub>2</sub>S. L-cystathionine is decomposed by CSE into L-cysteine, α-ketobutyrate and NH<sub>3</sub> by means of α, γ-elimination. L-cysteine continues to participate in the reaction. 4 and 5: L-cysteine is catalyzed to produce L-serine and H<sub>2</sub>S via CBS β-elimination or CSE α, β-elimination. 6 and 7: CBS catalyzes L-cysteine to produce pyruvate, NH<sub>3</sub> and H<sub>2</sub>S through α, β-elimination. 8 and 9: CSE first polymerizes two L-cysteines into L-cystathionine, then CSE uses L-cystathionine as a substrate to decompose it into thiocysteine (mercaptocysteine, Cys-SSH), pyruvate and NH<sub>3</sub>, resulting in thiocysteine generating H<sub>2</sub>S via nonenzymatic reactions with other thiols. 8: L-homocysteine generates α-ketobutyrate, NH<sub>3</sub> and H<sub>2</sub>S through CSE α, γ-elimination. In the mitochondria, CAT catalyzes L-cysteine and α-ketoglutarate to produce 3-MP, which is then catalyzed by 3-MST to produce pyruvate and H<sub>2</sub>S. In peroxisomes, 3-MP produced by DAO and catalyzed by D-cysteine is transported to the mitochondria in vesicles. H<sub>2</sub>S, hydrogen sulfide; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; 3-MP, 3-mercaptopyruvate; CAT, cysteine transaminase; 3-MST, 3-MP sulphotransferase; DAO, D-amino acid oxidase.

expression of aquaporin (AQP)-2 in the renal medulla. Following treatment with GYY4137, a H<sub>2</sub>S donor sustained release agent, expression levels of AQP-2 were significantly upregulated (30).

H<sub>2</sub>S can directly target some H<sub>2</sub>S-sensitive disulfide bonds in the epidermal growth factor receptor (EGFR), which can induce endocytosis and inhibition of Na-K-ATPase in renal tubular

epithelial cells by regulating the EGFR/GAB1/PI3K/Akt pathway, thus reducing sodium and potassium ion exchange of renal tubular epithelial cells, and promoting sodium excretion (31). However, how the EGFR/GAB1/PI3K/Akt pathway acts on Na-K-ATPase remains to be determined. EGFR is known to possess tyrosine kinase activity, and

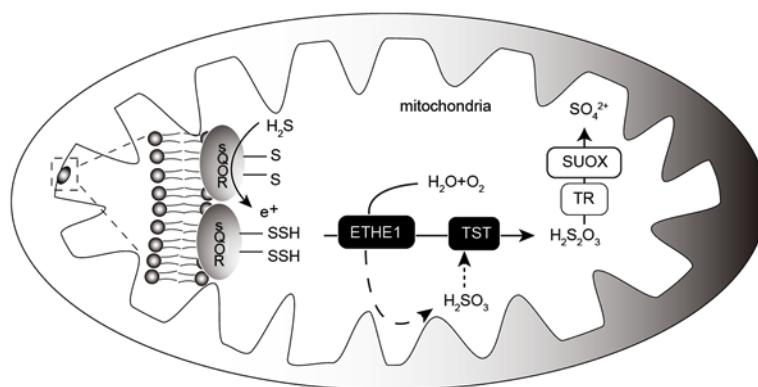


Figure 3. Oxidative metabolism of H<sub>2</sub>S in the mitochondria. H<sub>2</sub>S in the mitochondria is activated by SQOR, which receives an-SH group to form an-SSH group. In the presence of O<sub>2</sub> and H<sub>2</sub>O, -SSH is used by ETHE1 to generate H<sub>2</sub>SO<sub>3</sub>, which is further converted into thiosulfate by TST using the-SSH group. Finally, thiosulfate is oxidized by TR and SUOX, and is eventually excreted in the kidney as sulfate. H<sub>2</sub>S, hydrogen sulfide; SQOR, sulfoquinone oxidoreductase; ETHE1, thiodioxygenase; TST, thiosulfate sulfur transferase; TR, thiosulfate reductase; SUOX, sulfite oxidase.

its family members can bind to a variety of ligands to form homodimers or heterodimers, leading to the phosphorylation of specific tyrosine residues in intracellular domains. In renal vascular endothelial cells, inhibition of EGFR has been reported to dilate renal vessels and improve renal blood flow; in podocytes, inhibition of EGFR may reduce podocyte damage and loss induced by high glucose levels, and reduce proteinuria, whereas in renal tubular epithelial cells, inhibition of EGFR was shown to alleviate renal tubular injury and epithelial-mesenchymal transition (EMT) (32,33). However, studies on inhibitors of EGFR tyrosine kinase activity have shown that inhibition of EGFR can also lead to renal tubular damage and electrolyte disturbance (34). Therefore, more in-depth studies are required, particularly with regard to the advantages and disadvantages of H<sub>2</sub>S in regulating EGFR pathway activity.

Thus, these aforementioned previous studies indicated that H<sub>2</sub>S has a role in the metabolism of water and electrolytes via a variety of methods. In general, it has been suggested that the increased concentration of H<sub>2</sub>S is conducive to regulating the excretion of electrolytes by the kidney, whereas the inhibition of its production can preserve sodium drainage. Therefore, H<sub>2</sub>S-generating enzyme CBS and CSE inhibitors may be potential diuretics.

**Oxygen sensing.** H<sub>2</sub>S-mediated O<sub>2</sub> sensing has been detected in various O<sub>2</sub>-sensing tissues in the cardiovascular and respiratory systems of vertebrates (35,36). The effect of H<sub>2</sub>S on downstream signaling events is consistent with that of hypoxia activation (37,38). In normal kidneys, due to the intrarenal arteriovenous oxygen shunt, the kidney is in a state of low oxygen partial pressure compared with other organs, and the renal medulla oxygen partial pressure is lower than that of the renal parenchyma (39,40). Therefore, H<sub>2</sub>S is regarded as an oxygen sensor in the kidney, particularly in the medulla (41). As an oxygen sensor, H<sub>2</sub>S is inseparable from its generation and oxidative metabolic balance. H<sub>2</sub>S generation is not dependent on O<sub>2</sub>, but its oxidative metabolism in mitochondria is dependent on oxygen, as aforementioned; therefore, hypoxia can lead to an increase in H<sub>2</sub>S concentration and an inverse relationship exists between the two (37). The mitochondrial oxidative

respiratory electron transport chain is the primary means of energy generation; thus it is necessary and significant to prove that H<sub>2</sub>S participates in energy generation under physiological conditions in the renal medulla under normal hypoxia. As an oxygen sensor, H<sub>2</sub>S can affect the blood flow supply and regulate the oxygen balance in the heart and lungs. Whether H<sub>2</sub>S also regulates the distribution of oxygen supply in the renal cortex and medulla under physiological conditions through this mechanism or via other means remains to be determined. Investigating the location and molecular mechanism of H<sub>2</sub>S as an oxygen sensor affecting the occurrence of downstream signaling events will further enrich our understanding of H<sub>2</sub>S as an oxygen sensor.

## 5. Role of H<sub>2</sub>S in renal disease

**Renal injury.** Our previous study revealed that the expression levels of CBS and CSE, two enzymes that produce H<sub>2</sub>S, were decreased in renal tissues following urinary tract obstruction (42). *In vivo* studies also demonstrated that supplementation with an H<sub>2</sub>S donor, to provide sufficient H<sub>2</sub>S, improved renal injury (42); the mechanisms and molecular pathways involved are relevant to the disease model studied. Kidney injury can be divided into two categories: Acute kidney injury (AKI) and CKD. AKI may occur as a result of ischemia-reperfusion (hemorrhagic or septic shock) or after exposure to toxic substances (such as iodized contrast agents, aminoglycosides and cisplatin). CKD occurs in glomerular and tubular interstitial lesions, such as diabetic nephropathy (DN) and hypertensive nephropathy, amongst other causes (43).

**Ischemia-reperfusion injury (IRI).** In the process of kidney transplantation, the temporary cessation of renal blood flow leads to acute ischemic injury, and reperfusion further enhances the functional and structural damage to human kidneys, namely renal ischemia-reperfusion injury (IRI). Animal experiments have shown that following renal ischemia-reperfusion, serum and tissues exhibit markedly increased levels of IL and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), alongside other inflammatory indicators, significantly elevated malondialdehyde (MDA) concentrations, significantly reduced superoxide dismutase

(SOD) activity and renal tubular necrosis; conversely, the  $\text{H}_2\text{S}$  donor  $\text{Na}_2\text{S}$  has been shown to significantly reduce inflammation, oxidative stress and kidney damage, as shown in Fig. 4 (44). Increased levels of MDA and reduced activity of SOD have been shown to promote lipid peroxidation and upregulate nuclear factor- $\kappa\text{B}$  (NF- $\kappa\text{B}$ ), IL-2 and Toll-like receptor-4 (TLR-4), which can stimulate an inflammatory response, thereby increasing renal cell apoptosis (45). The CSE inhibitor, propargyl glycine, or the CBS inhibitor, hydroxylamine, have been shown to aggravate AKI and apoptosis, presenting with higher levels of pro-inflammatory factors, significantly increased levels of NF- $\kappa\text{B}$  (P65), and phosphorylated (p)-apoptosis signal-regulating kinase 1 and p-TNFR-associated factor 2. These changes were accompanied by the increased expression levels of TLR-2 and TLR-4, indicating that a TLR-mediated inflammatory response and apoptosis are also involved in renal IRI (46).

The mitochondrial targeted  $\text{H}_2\text{S}$  donor, AP39, has been reported to significantly improve the survival and function of donor kidney transplantation, and reduce cell apoptosis and necrosis (47,48).  $\text{H}_2\text{S}$  has been shown to attenuate apoptosis and necrosis during cryopreservation of a donor kidney, and may increase the survival rate and function of transplanted kidneys by regulating the mitochondrial membrane potential and reducing reactive oxygen species (ROS) production (47). Oxidative stress induced by glucose oxidase can lead to mitochondrial dysfunction, which reduces the levels of ATP in renal epithelial cells, increases the formation of cellular ROS at a relatively high concentration and promotes cell necrosis. A previous study using both *in vitro* and *in vivo* experiments found that AP39 pretreatment has a concentration-dependent protective effect on renal IRI, with the most significant effect observed at a concentration of  $300 \text{ nmol l}^{-1}$  (48). The  $\text{H}_2\text{S}$  protection of AP39 was 1,000X higher than that of GYY4137, a nonspecific exogenous  $\text{H}_2\text{S}$  donor (47). In addition,  $\text{H}_2\text{S}$  may reduce the inflammatory response by inhibiting activation of the Nod2 signaling pathway and suppressing the type A macrophage scavenger receptor signaling pathway to upregulate endoplasmic reticulum stress-induced autophagy to protect the kidney from IRI (49). However, how  $\text{H}_2\text{S}$  acts on these targets is unclear.

Studies on renal transplantation storage also demonstrated that long-term static storage of donation after cardiac death (DCD) kidneys at  $21^\circ\text{C}$  in UW solution supplemented with AP39 may increase the activity of renal tubular epithelial cells and reduce tissue necrosis compared with long-term static storage at  $4^\circ\text{C}$  in UW solution. However, the experimental results also revealed that the UW solution supplemented with AP39 exhibited improved cell-protective effects at  $4^\circ\text{C}$  compared with that at  $21^\circ\text{C}$  (50). This is consistent with static cryogenic storage (SCS) and continuous cryogenic machine perfusion commonly used in our clinic. However, it is worth noting that organ preservation at a physiological temperature ( $37^\circ\text{C}$ ), such as normal temperature machine perfusion, may be worthy of study to better prevent the damage of transplanted organs caused by low temperatures (51). Renal function was revealed to be improved in transplanted kidneys stored at a normal physiological temperature compared with those stored in the cold state (52). In a previous study, kidneys from expanded criteria donors (ECD) were normally perfused

*in vitro* for  $63 \pm 16$  min with a plasma-free red cell-based solution at an average temperature of  $34.6^\circ\text{C}$  and compared with 47 ECD kidneys with CSC in a control group; the results showed that all donor kidneys were successfully transplanted with good renal function (53). In addition, subnormothermic machine perfusion of DCD porcine grafts at  $20^\circ\text{C}$  has been shown to improve graft prognosis compared with hypothermic machine perfusion and SCS (54). Therefore, the effects of  $\text{H}_2\text{S}$  and the storage temperature of transplanted kidneys should be studied further to determine the ideal storage conditions.

**Drug nephrotoxicity.** Cisplatin is a common chemotherapeutic drug that is widely used in the clinic. Cisplatin, by downregulating the expression levels of CSE, is known to disrupt  $\text{H}_2\text{S}$  generation and lead to the death of proximal tubular cells, thereby causing renal toxicity. The  $\text{H}_2\text{S}$  donors, NaHS and GYY4137, have been reported to reduce cisplatin-induced cell death and renal toxicity (55). A previous study revealed that  $\text{H}_2\text{S}$  can increase S-sulphydrylation of the Cys256, Cys259, Cys280 and Cys283 residues of NAD-dependent deacetylase sirtuin-3 (SIRT3), which induces the deacetylation of its target proteins, dynamin-like 120 kDa protein (OPA1), ATP synthase and SOD2, thus reducing mitochondrial division and increasing ATP production, and thereby reducing oxidative damage (56). In addition,  $\text{H}_2\text{S}$  may inhibit the generation of intracellular ROS and MAPKs by inhibiting the activity of NADPH oxidase, which is related to the vulcanization effect of  $\text{H}_2\text{S}$  on the NADPH oxidase subunit P47PHOX (55). Whilst reducing NADPH oxidase activity,  $\text{H}_2\text{S}$  may also induce nuclear translocation of the nuclear factor erythroid 2-related factor 2 (Nrf2) to inhibit the production of ROS in cells. Further experiments have revealed that exogenous  $\text{H}_2\text{S}$  donors lead to the phosphorylation of Akt and dimerization of Kelch-like ECH-related protein 1 (Keap1); the inhibition of Akt activation has been reported to not only weaken the nuclear translocation of Nrf2, but also reduce the protective effects of exogenous  $\text{H}_2\text{S}$  donors (57).  $\text{H}_2\text{S}$  can activate Nrf2 translocation to the nucleus by dimerizing Keap1, thus promoting the expression of antioxidant genes (58). Therefore,  $\text{H}_2\text{S}$  is hypothesized to inhibit ROS production in cells via Akt/Keap1 and the activation of MAPKs, thereby mediating the nuclear translocation of Nrf2. It may also inhibit the production of ROS in cells by reducing the activity of NADPH oxidase. Recent studies have shown that exogenous  $\text{H}_2\text{S}$  serves a renoprotective role in cyclophosphamide-induced nephrotoxicity, which is associated with increased expression of Nrf2 and downstream antioxidant proteins, such as heme oxygenase-1 (HO-1), and reduced glutathione and SOD in renal tissues (57,59), as shown in Fig. 4.

The histopathological results of a previous study revealed that the renal tissues of a cisplatin group were positive for desmin protein expression, with notable podocyte injury, increased quantities of mesangial matrix and increased proliferation of mesangial cells. Notably, NaHS therapy could improve podocyte injury and increase nephrin protein levels (60). These findings suggested that  $\text{H}_2\text{S}$  may improve cisplatin-induced renal injury by protecting renal podocyte cells. In gentamicin-induced kidney injury in rats, NaHS significantly reduced renal NO and TNF- $\alpha$  levels, whilst increasing total antioxidant capacity (T-AOC), HO-1 and



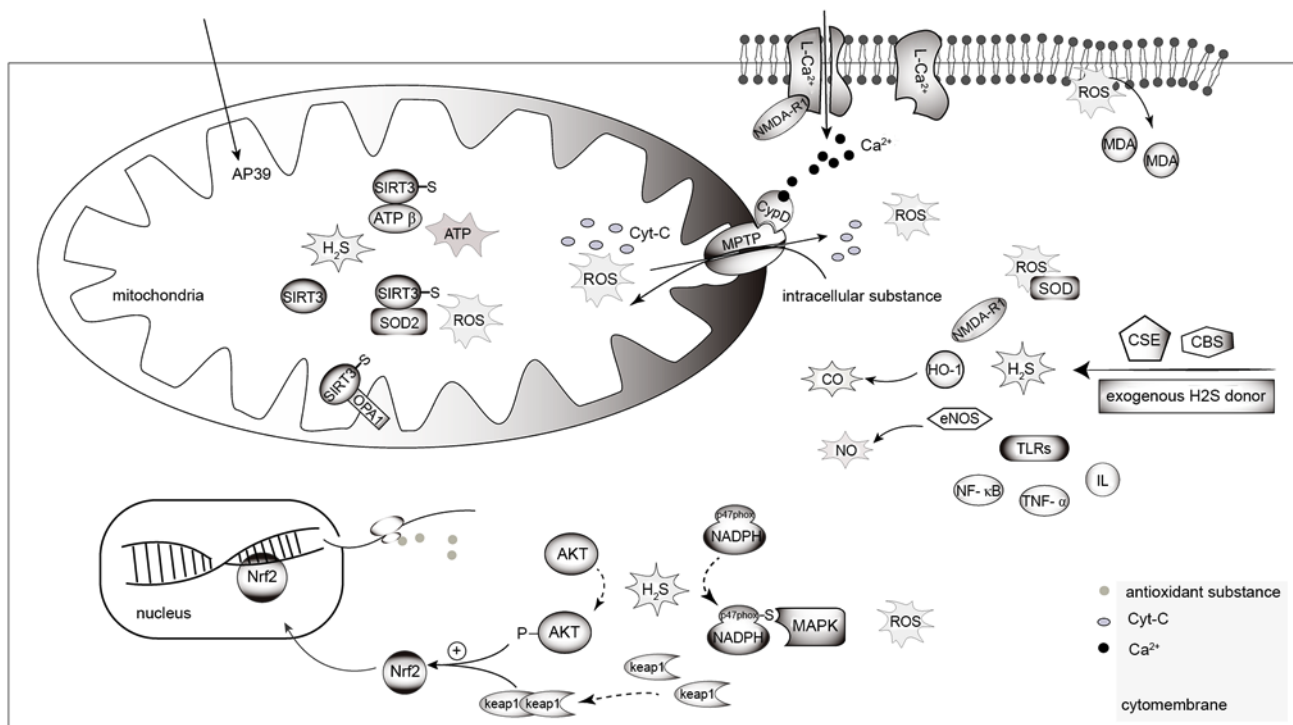


Figure 4. H<sub>2</sub>S and renal injury. In the mitochondria, H<sub>2</sub>S increases S-mercaptoylation of the four cysteine residues of SIRT3, and induces deacetylation of its target proteins, OPA1, ATP synthase (depicted as ATP in the figure) and SOD2, thus reducing mitochondrial division and increasing ATP production. H<sub>2</sub>S reduces MPTP opening and loss of mitochondrial membrane potential via Ca<sup>2+</sup>-dependent CypD activation by inhibiting NMDA-R1 mediated Ca<sup>2+</sup> influx, thus avoiding mitochondrial morphological and functional damage, leading to ROS accumulation. ROS leads to the peroxidation of membrane lipids to MDA, causing damage to cells and organelles. The prevulcanization of H<sub>2</sub>S on NADPH oxidase subunit p47PHOX inhibits the activity of NADPH oxidase, thereby inhibiting the generation of MAPKs and intracellular ROS. H<sub>2</sub>S leads to the phosphorylation of AKT and dimerization of Keap1, and induces nuclear translocation of Nrf2 to promote the expression of antioxidant substances, thereby inhibiting ROS in cells. NaHS downregulates the overexpression of renal iNOS, upregulates eNOS and HO-1, and regulates the T-AOC and IL-10 via the CO/NO pathway to exert an anti-inflammatory and antioxidant effect. H<sub>2</sub>S, hydrogen sulfide; OPA1, dynamin-like 120 kDa protein; SIRT3, NAD-dependent deacetylase sirtuin-3; SOD2, superoxide dismutase 2; MPTP, mitochondrial permeability transition pore; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; HO-1, heme oxygenase-1; T-AOC, total antioxidant capacity; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-related protein 1; ROS, reactive oxygen species; TLR, Toll-like receptor; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; p-, phosphorylated; MDA, malondialdehyde; CO, carbon monoxide; NO, nitric oxide.

IL-10 levels, and reduced the increase in renal inducible NOS (iNOS), whilst upregulating eNOS levels. Zinc proporphyrin (a selective HO-1 inhibitor) could reverse these changes, and block the anti-inflammatory and antioxidant effects of H<sub>2</sub>S (61). Therefore, H<sub>2</sub>S may serve an anti-inflammatory and antioxidant role in protecting AKI, partly by relying on the CO/NO pathway, and this mechanism may function to primarily downregulate NO levels, or to downregulate the effects of NO by increasing CO levels (61).

**DN.** Streptomycin-induced DN rats have been shown to exhibit notable inflammation and oxidative stress, with obvious renal decline and insufficiency, decreased activities of SIRT1 and SOD, and increased relative expression of caspase-3, p53 and MDA; however, NaHS may improve renal function, manifested as significantly reduced serum urea and creatinine levels, and markers of renal injury, and reversal of the aforementioned indicators of DN (62,63). ATP-sensitive potassium (K<sub>ATP</sub>) channels and L-type calcium channels have been shown to be related to the increase in ROS levels and oxidative stress in DN renal cells. NaHS may increase T-AOC and reduce the total NO levels in a rat model of DN, and the use of K<sub>ATP</sub> inhibitors may further increase T-AOC and reduce NO levels (62). Therefore, the renoprotective mechanism

of H<sub>2</sub>S on DN may be partly dependent on K<sub>ATP</sub> channel activation-mediated effects on renal tissue antioxidants and NO.

In a previous study, renal injury was simulated in C57BL/6J and Akita (C57BL/6JIns2Akita) mice under a high-glucose environment, and the experiment showed increased cytoplasmic Ca<sup>2+</sup> influx, activation of the mitochondrial matrix protein cyclophilin D (CypD), increased mitochondrial permeability transition opening, loss of mitochondrial membrane potential and oxidative burst. The H<sub>2</sub>S donor GYY4137 could reduce the aforementioned effects following treatment. Similar results were also observed with the N-methyl-D-aspartate receptor-R1 (NMDA-R1) blocker MK-801, which further confirmed that H<sub>2</sub>S function may involve NMDA-R1 (64). H<sub>2</sub>S has been reported to reduce intracellular Ca<sup>2+</sup> by inhibiting NMDA-R1-mediated inflow of Ca<sup>2+</sup> ions, and thus reducing Ca<sup>2+</sup>-dependent CypD activation to result in mitochondrial permeability transition pore opening and loss of mitochondrial membrane potential. This effects may avoid damage to the mitochondrial morphology and function, and could cause outbreaks of active oxygen substances and protect diabetic kidney cells from oxidative stress injury, as shown in Fig. 4. In dose-response experiments assessing the protective effects of H<sub>2</sub>S on DN kidney, it was

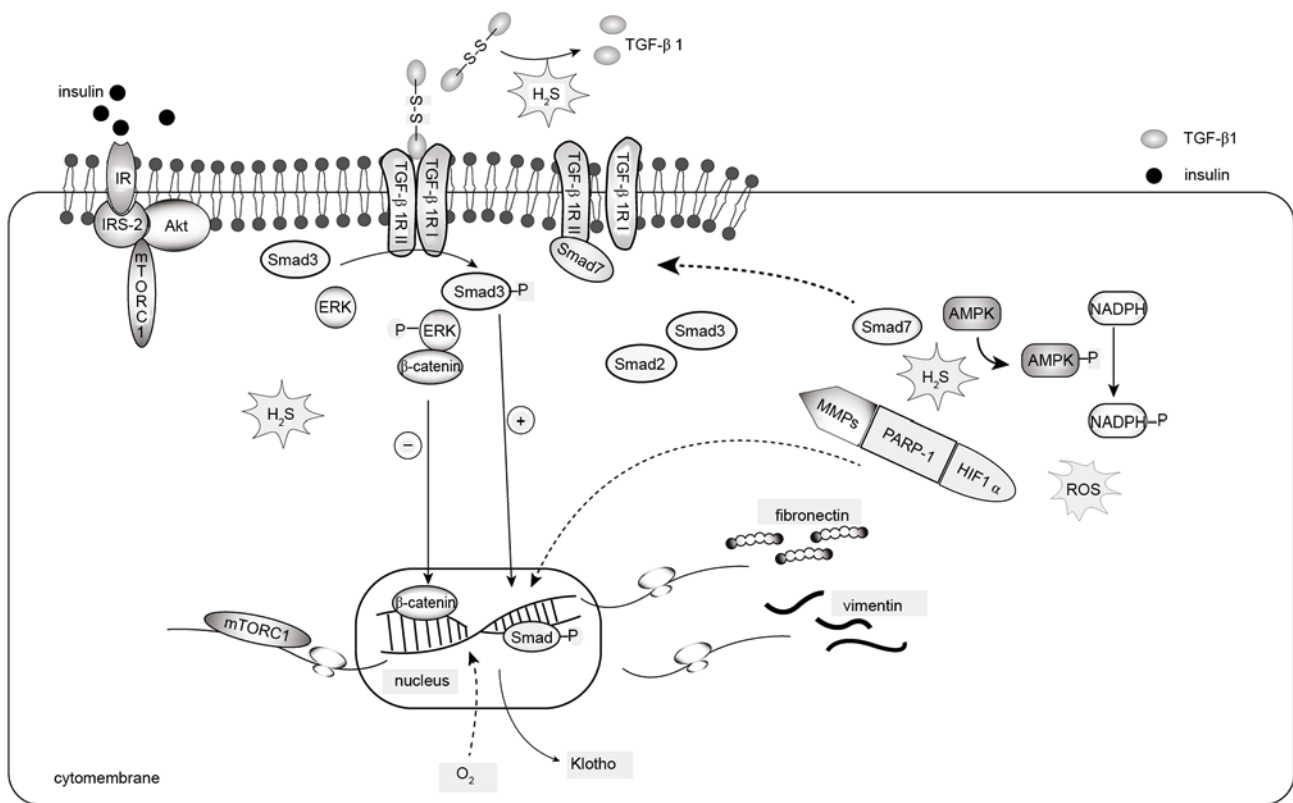


Figure 5. H<sub>2</sub>S and renal fibrosis. TGF-β1 binds to TβR and promotes downstream Smad protein activation, leading to the overexpression of fibronectin and vimentin. Activated by TβR, ERK promotes the conversion of β-catenin in the nucleus, leading to the increased expression of fibronectin. H<sub>2</sub>S promotes Smad7 expression to reduce the combination of TβRII and TβRI, preventing this process. At the same time, H<sub>2</sub>S lyses the disulfide bond in the active TGF-β1 dimer, promoting the formation of inactive TGF-β1 monomers. In addition, increases in the expression of matrix-associated proteins are associated with the activation of the IR/IRS-2/Akt-mTORC1/mRNA transcriptional signaling axis. H<sub>2</sub>S reduces ROS and collagen cross-linking by regulating MMPs/PARP-1/HIF-1. Hypoxia is associated with methylation and expression silencing of the Klotho promoter. H<sub>2</sub>S can significantly improve hypoxia, reverse Klotho promoter methylation and increase Klotho expression. TGF-β1, transforming growth factor-β1; TβR, TGF-β receptor; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; H<sub>2</sub>S, hydrogen sulfide; IR, insulin receptor; IRS, IR substrate; mTORC, mammalian target of rapamycin complex 1; MMP, matrix metalloproteinase; PARP, poly ADP-ribose-polymerase; HIF-1, hypoxia-inducible factor-1; p-, phosphorylated.

found that at a dose of 100 mol/kg/day, the activity/expression of SIRT1 returned to normal, and the kidney function of DN rats was improved (63). However, this previous study did not elaborate on the relationship between SIRT1 and oxidative stress and inflammation, and the molecular mechanism of action between them still remains to be further explored.

**Renal fibrosis.** Long-term damage to the kidney by various factors can lead to the occurrence of renal fibrosis. In the kidney of diabetic rats, NaHS therapy downregulated the expression of transforming growth factor-β1 (TGF-β1), extracellular signal regulated kinase 1/2 (ERK1/2), tissue inhibitors of metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs), leading to the improvement of renal fibrosis (65,66). Renal fibrosis is associated with TGF-β/Smad signaling, AMP-activated protein kinase (AMPK) activation, ERK1/2 expression and MMP/TIMP dysregulation (65,66), as shown in Fig. 5.

The novel H<sub>2</sub>S-releasing compound, S-propylcysteine, was revealed to inhibit the mRNA expression levels of hyperglycemic fibulin and type IV collagen, as well as the over-proliferation and hypertrophy of mesangial cells. Further experiments confirmed that this was related to the inhibition of TGF-β1- and Smad3-related signaling pathways (67).

Following unilateral ureteral obstruction (UO) in male Lewis rats, H<sub>2</sub>S treatment was shown to reduce serum creatinine and urine protein/creatinine excretion rate, and tissues exhibited reduced expression of EMT-related proteins, including fibronectin, vimentin, Smad2, TGF-β1 and TGF-β1 receptor (TβR)II. Pathological analysis also showed that H<sub>2</sub>S alleviated cortical loss, inflammatory damage and renal tubulointerstitial fibrosis (68). Previous studies have shown that H<sub>2</sub>S-mediated Smad7 expression may reduce TβRII expression, and improve UO renal fibrosis in a rat model via the upregulation of cadherin expression and downregulation of vimentin expression in endothelial cells (69-71). In this mechanism, TβRII binds to and activates TβRI, which can increase the activation of downstream Smad expression, leading to the upregulation of vimentin expression and downregulation of cadherin expression in endothelial cells; Smad7 can interact with TβRI/TβRII to prevent this process (70,71). *In vitro* experiments using human recombinant active TGF-β1 to induce EMT also found that H<sub>2</sub>S cleaved the disulfide bonds in the active dimer of TGF-β1, and promoted the formation of inactive TGF-β1 monomers (72). In addition, NaHS reduced the increase in expression of β-catenin induced by TGF-β1, increased the phosphorylation of ERK and inhibited the nuclear translocation of β-catenin induced by TGF-β1. Using the ERK

inhibitor U0126 or  $\beta$ -catenin small interfering RNA (siRNA) agent XAV939 abrogated the effects of NaHS on fibronectin, E-cadherin and TGF- $\beta$ RI. These findings indicated that H<sub>2</sub>S may block TGF- $\beta$ -induced EMT by inhibiting ERK activation and  $\beta$ -catenin translocation, thus preventing renal fibrosis (73).

In diabetic Akita mice, the levels of plasma H<sub>2</sub>S, ROS and its regulator ROS modulator 1, and the expression of collagen cross-linking proteins (prolyl 4-hydroxylase subunit  $\alpha$  1 and procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2) were increased, and the activity and the expression levels of poly ADP-ribose-polymerase-1 (PARP-1), hypoxia-inducible factor-1 (HIF-1), and MMP-9, -13 and -14 were increased. These findings may be related to the downregulation of microRNA (miR)-194. Notably, GYY4137 was shown to restore expression of miR-194. In addition, *in vivo* and *in vitro* experiments revealed that cells transfected with miR-194 mimic exhibited alleviation of high glucose-induced ROS production (74). A high-glucose environment may increase ROS levels and lead to PARP activation, whereas PARP-1 deficiency may alleviate DN (75). Furthermore, blocking HIF-1 may reduce glomerular hypertrophy, ECM deposition and urinary albumin excretion in diabetic kidneys (76). These results suggested that H<sub>2</sub>S may alleviate diabetic renal ECM deposition and thereby reduce renal fibrosis by regulating MMPs/PARP-1/HIF-1 expression to reduce ROS levels and the increase in collagen cross-linking.

The increase in matrix protein content involved in renal fibrosis has been reported to be associated with AMPK activity and activation of the insulin receptor (IR)/IR substrate (IRS)-2/Akt/mammalian target of rapamycin complex 1 (mTORC1)/mRNA transcriptional signaling axis (77). In proximal renal tubular epithelial cells, high glucose levels inhibited AMPK phosphorylation and activity, increased NADPH oxidase 4 (NOX4) expression and activity, and the production of ROS and matrix protein synthesis, which was reversed by NaHS. In further experiments, an AMPK inhibitor prevented NaHS from reducing the expression of NOX4 induced by high glucose (78). In addition, it was revealed that N ( $\omega$ )-nitro-L-arginine methyl ester (a NOS inhibitor) could abolish NaHS inhibition of NOX4 expression induced by high glucose. NaHS enhanced the expression of iNOS instead of eNOS. Further experiments showed that iNOS siRNA and 1400W (a selective iNOS inhibitor) eliminated the favorable effects of NaHS on the expression of high glucose-induced NOX4, ROS and matrix laminin expression (78). Therefore, NaHS may regulate oxidative stress and the expression of renal interstitial matrix protein by inducing NO production and mediating the AMPK pathway to inhibit hyperglycemic renal fibrosis and protect diabetic renal function. Two gas transmitters, H<sub>2</sub>S and NO, and their interactions can be used as therapeutic targets for DN (78).

Hypoxia and inflammation can lead to renal fibrosis, and renal hypoxia is associated with methylation and silencing of the Klotho promoter. Notably, NaHS treatment has been reported to significantly reduce hypoxia, reverse Klotho promoter methylation to increase Klotho expression, and thereby improve renal tubular interstitial fibrosis in mice (79). Inhibition of M1/M2 macrophage infiltration and NLRP3 inflammasome activation, and subsequent inactivation of the NF- $\kappa$ B and IL-4/STAT6 signaling pathways can also exert anti-inflammatory and anti-fibrotic roles in the

protection against renal fibrosis and renal injury following obstruction (80). In renal tubular epithelial cells, H<sub>2</sub>S has been shown to sulfurize the two conserved domains of SIRT1 (Cys371/374 and Cys395/398), and induce the dephosphorylation and deacetylation of its target proteins NF- $\kappa$ B (p65) and STAT3, thereby reducing oxidative stress, inflammation and EMT caused by high glucose (81). Renal fibrosis is also associated with aging and obesity. Notably, NaHS restored AMPK activity, inhibited activation of the IR/IRS-2/Akt/mTORC1/mRNA translation axis, and improved renal function in aged mice (77). In addition, miR-21 has been shown to be associated with renal injury in the elderly. After inhibition of miR-21 expression, the expression levels of H<sub>2</sub>S-generating enzymes, CBS and CSE, in mouse endothelial cells were upregulated, and the expression levels of MMP-9 and type IV collagen were downregulated (82). In a high-fat diet (HFD)-induced model of kidney injury, H<sub>2</sub>S reduced the phosphorylation levels of IR and Akt in the renal cortex of male mice, which may suggest that obesity-related kidney injury is related to the IR/Akt pathway; however, this association was not observed in female mice, and whether it is related to sex-related factors remains to be studied (83). Furthermore, H<sub>2</sub>S significantly reduced lipid accumulation in the kidneys of HFD-induced obese mice, and studies have shown that H<sub>2</sub>S may downregulate NF- $\kappa$ B (P65) expression to reduce renal inflammation and alleviate HFD-induced renal injury in obese mice (84). These findings suggested that obesity may aggravate inflammatory-mediated renal fibrosis and renal injury.

## 6. Conclusions

H<sub>2</sub>S deficiency is a potential risk factor for the development and progression of renal diseases. A variety of renal injuries, including IRI, drug nephrotoxicity and DN, exhibit metabolic imbalances of H<sub>2</sub>S during their pathological development. Supplementing exogenous H<sub>2</sub>S can alleviate renal injury caused by these diseases, delay the progression of renal fibrosis and improve renal function. The signaling pathways and molecules in which H<sub>2</sub>S serves an antioxidant, anti-inflammatory, anti-apoptotic and anti-fibrotic role in renal protection are being increasingly better understood. In addition, organelles serve a notable role in the progression of AKI and CKD. At present, studies on the damage to organelles, such as mitochondrial homeostasis, mitochondrial autophagy and endoplasmic reticulum oxidative stress, are relatively limited with regard to the pathological mechanism of AKI and CKD, and more in-depth studies are required. In terms of exogenous H<sub>2</sub>S donors, research into drugs targeting the mitochondria and inducing the controlled release of agents may form an indispensable means of treatment for AKI and CKD.

## Acknowledgements

Not applicable.

## Funding

No funding was received.



## Availability of data and materials

Not applicable.

## Authors' contributions

NG conceived the idea of the present review, and was responsible for reviewing the images and main text. HuZ and HaZ were major contributors to the writing of the manuscript, and designed the figures. All authors read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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