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Implications of hydrogen sulfide in liver pathophysiology: Mechanistic insights and therapeutic potential

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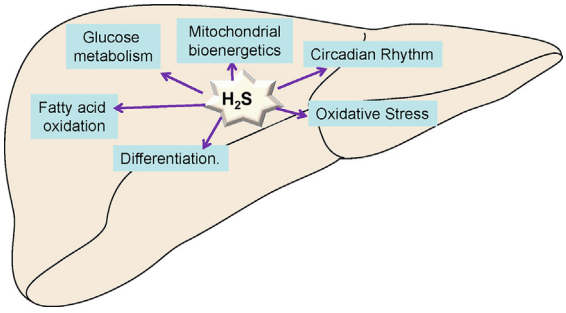
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Over the last several decades, hydrogen sulfide (H_2S) has been found to exert multiple physiological functions in mammal systems. The endogenous production of H_2S is primarily mediated by cystathione β-synthase (CBS), cystathione γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). These enzymes are widely expressed in the liver tissues and regulate hepatic functions by acting on various molecular targets.

Aim of Review: In the present review, we will highlight the recent advancements in the cellular events triggered by H_2S under liver diseases. The therapeutic effects of H_2S donors on hepatic diseases will also be discussed.

Key Scientific Concepts of Review: As a critical regulator of liver functions, H_2S is critically involved in the etiology of various liver disorders, such as nonalcoholic steatohepatitis (NASH), hepatic

Abbreviations: 3-MP, 3-mercaptopyruvate; 3-MST, 3-mercaptopyruvate sulfurtransferase; AGTR1, angiotensin II type 1 receptor; Akt, protein kinase B; AMPK, AMP-activated protein kinase; CAT, cysteine aminotransferase; CBS, cystathione β-synthase; CO, carbon monoxide; CSE, cystathione γ-lyase; COX-2, cyclooxygenase-2; CX3CR1, chemokine CX3C motif receptor 1; DATS, Diallyl trisulfide; DAO, D-amino acid oxidase; EGFR, epidermal growth factor receptor; ERK, extracellular regulated protein kinases; FAS, fatty acid synthase; H_2S, hydrogen sulfide; HFD, high fat diet; HO-1, heme oxygenase 1; IR, ischemia/reperfusion; MMP-2, matrix metalloproteinase 2; mTOR, mammalian target of rapamycin; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, non-alcoholic fatty liver diseases; NF-κB, nuclear factor-kappa B; NaHS, sodium hydrosulfide; NASH, nonalcoholic steatohepatitis; Nrf2, nuclear factor erythroid2-related factor 2; PI3K, phosphatidylinositol 3-kinase; PLP, pyridoxal 5′-phosphate; PPG, propargylglycine; PTEN, phosphatase and tensin homolog deleted on chromosome ten; SAC, S-allyl-cysteine; SPRC, S-propargyl-cysteine; STAT3, signal transducer and activator of transcription 3; VLDL, very low density lipoprotein.

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Fibrosis
Cancer

ischemia/reperfusion (IR) injury, and liver cancer. Targeting H_2S-producing enzymes may be a promising strategy for managing hepatic disorders.

Introduction

Hydrogen sulfide (H_2S) is previously described as a toxic gas for a long time [1]. However, mounting evidence suggests its critical roles in numerous biological functions, especially in the cardiovascular [2,3], central nervous [4–6], and other systems [7–10]. It has been proposed that endogenous H_2S in mammal systems is mainly generated by either enzymatic or non-enzymatic pathways. The enzymatic process is dependent on the actions of cystathione β-synthase (CBS), cystathione γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [11–13]. The non-enzymatic pathway involves two pathways: the reduction of elemental sulfur generated by the intermediate reduction product of oxidized glucose in the process of glycolysis and the phosphogluconate pathway in erythrocytes [14,15]. Over the last several decades, a pathological significance of H_2S has enormously grown in the development of several liver ailments [16]. As a consequence, research on H_2S-governed liver functions has achieved considerable progression in both health and diseases. Nevertheless, the cellular and molecular mechanisms that underlie H_2S-mediated liver functions have not been fully clarified. Considering that H_2S is critically involved in numerous biological processes, it is now extensively accepted that H_2S serves as a principal mediator in the field of biological gases.

It is well known that the liver is involved in glycolipid metabolism, xenobiotic metabolism, and host defenses against invading microorganisms [16–20]. Importantly, the liver is a key organ for the production and clearance of H_2S [21]. Studies have shown that CBS, CSE and 3-MST are responsible for H_2S generation in the liver [16]. A host of genes are engaged in glycolipid metabolism, mitochondrial biogenesis and bioenergetics in which H_2S plays a critical role [16,22–24]. Of importance, H_2S production and signaling in the liver are altered in several hepatic diseases, including hepatic ischemia/reperfusion (I/R) injury [25], nonalcoholic steatohepatitis (NASH) [26], liver fibrosis [27], and liver cancer [28]. As continuous research into the roles of H_2S in the control of liver health and diseases, the potential mechanisms of H_2S-mediated liver protection have started to be elucidated. In this review, we will overview the present studies of H_2S in the context of liver diseases, with special emphasis on the mechanistic insights and therapeutic potential of H_2S in several liver diseases.

Production of H_2S in the liver

H_2S is an endogenous gasotransmitter that is mainly produced via both enzymatic and non-enzymatic reactions, and it can also be generated from intracellular sulfur stores [16,29]. In the process of the enzymatic pathway, as two pyridoxal 5′-phosphate (PLP)-dependent enzymes, CSE and CBS lead to H_2S generation using L-cysteine and homocysteine as major substrates [30,31]. Different from CBS and CSE, PLP-independent 3-MST gives rise to H_2S by using 3-mercaptopyruvate (3-MP) as a principal substrate. 3-MP is an intermediate metabolite from either L-cysteine or α-ketoglutarate with the aid of cysteine aminotransferase (CAT) [31]. The reactions for H_2S production are summarized in Fig. 1. CSE activity is much higher than CBS in peripheral tissues, whereas CBS is mainly distributed in the brain for primary H_2S production [15]. CSE is majorly responsible for H_2S generation in the cardiovascular system, while CBS is a predominantly expressed enzyme in the brain tissues [32]. 3-MST is localized in both cytosol and mitochondria, but the majority of 3-MST is distributed in the mitochondria [33,34], as the concentration of L-cysteine in the mitochondria is three times higher than that in the cytoplasm [35].

By using a non-enzymatic system, endogenous H_2S is also produced by glucose, inorganic and organic polysulfides, glutathione, and sulfane sulfur [9,36–38]. In general, H_2S could be produced from the reducing equivalents, including nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) that are generated through glycolysis-mediated glucose oxidation or NADPH oxidase-mediated phosphogluconate oxidation [14]. Reactions of glucose with methionine, cysteine, or homocysteine may lead to the production of H_2S and methanethiol [36,39,40]. In the process of non-enzymatic chemical reduction, the intermediate products of sulfur metabolism are available in mammal cells, indicating the necessity of such pathway for H_2S production in mammalian systems. Moreover, garlic and garlic-derived organic polysulfides may be an important source for H_2S generation, including diallyl trisulfide, diallyl disulfide, diallyl sulfide and S-allyl cysteine [38,41–43]. Accordingly, it is highly probable that the non-enzymatic pathway is a key supplement for H_2S generation in the body. However, more studies are still warranted to identify the physiological significance of such non-enzymatic pathway in H_2S production in mammalian systems.

In the liver, although the three H_2S-generating enzymes are detectable, their roles in endogenous H_2S generation are differently described [44–46]. It is found that CSE expression is about 60-fold more than CBS in the liver [45], this observation is supported by a finding that genetic knockout of CSE diminishes the majority of H_2S production in the liver, further confirming that CSE is a primary enzyme for H_2S generation in the liver tissues [32,47]. Intriguingly, knockdown of 3-MST stimulates H_2S production, whereas overexpression of 3-MST markedly inhibits the formation of H_2S [26], implying the negative role of 3-MST in endogenous liver H_2S production. In addition, the cell-type heterogeneity of these three enzymes in liver tissues points to the sophisticated actions of H_2S-producing enzymes on the liver functions. The expression levels of CBS, CSE, and 3-MST are observed in hepatocytes [48]. As described previously, CSE protein is expressed in hepatic stellate cells, while CBS is not detected [47]. In the same study, CSE and CBS are not found in sinusoidal endothelial cells, at least in rats [47]. Until now, the expression of 3-MST is not available in these three non-parenchymal liver cells. It is likely that the H_2S-producing enzymes show cell-type specific regulation in the liver. Since the distinct cell types contribute to different liver functions, determination of cell-type specific production of H_2S in the liver system is indispensable for the elucidation of endogenous H_2S-mediated hepatic functions.

As mentioned above, the enzymes for H_2S production are largely observed in the liver tissues. Additional work is needed to clarify the roles of endogenous H_2S in liver heath and diseases. In earlier reviews, accumulating evidence has demonstrated a close relationship between H_2S and normal hepatic functions [10,13,16,23,49–58]. In this review, we will highlight recent studies regarding the potential roles and mechanisms of H_2S in several liver disorders, such as I/R injury in the liver, hepatic fibrosis, NASH, and liver cancer.

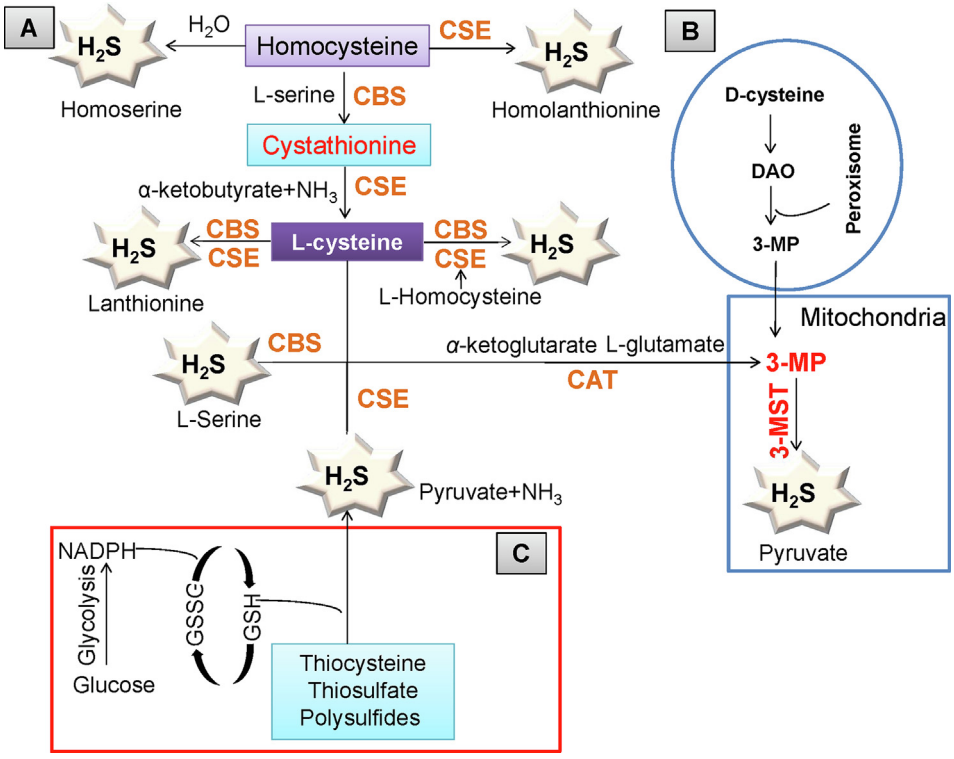

Fig. 1. The generation pathways of H_2S. (**A**) H_2S is generated from L-cysteine and homocysteine with the aid of CSE and CBS. (**B**) Peroxisome-induced production of 3-MP from D-cysteine by using DAO. Then, 3-MP is transferred into the mitochondria and serves as a supplement for 3-MST to yield H_2S. (**C**) H_2S is also produced by non-enzymatic reaction. The persulfides, thiosulfate, and polysulfides could be transformed into H_2S and other additional products in the presence of NADPH and NADH.

Role of H_2S in liver I/R injury

I/R is reflected by initial deprivation of blood in an organ or a specific area, followed by restoration of blood and re-oxygenation [59]. I/R-induced organ or tissue injury leads to increased morbidity and mortality in various pathologies, such as ischemic stroke, trauma, myocardial infarction, acute kidney injury, sickle cell disease, organ transplantation, and bypass surgery [60]. Similarly, liver I/R-elicited injury contributes to profound liver damage and ultimate mortality [61]. Hepatic I/R injury is involved in the pathogenesis of numerous clinical entities after hepatic surgery and transplantation [61,62]. A host of studies have demonstrated that the pathogenesis of hepatic I/R injury may involve intracellular anaerobic metabolism, overproduction of inflammatory cytokines, oxidative stress, mitochondria dysfunction, and activation of immune cells, such as Kupffer cells and neutrophils [63,64]. Despite that the recent progresses in surgical techniques and perioperative cares have been achieved, liver I/R injury is still recognized as one of the most complications in liver resection surgery, trauma, transplantation, and hypovolemic shock [63,65]. As a result, it is pressing to identify effective therapeutic strategies to attenuate or prevent hepatic injury induced by I/R.

A large body of evidence has demonstrated that H_2S is effective in protecting the liver from I/R injury, thereby representing a novel avenue to reduce the rate of morbidity and mortality triggered by hepatic I/R injury [25,66–68]. In liver I/R injury rats, the expression levels of CSE and H_2S are upregulated, whereas administration of H_2S donor sodium hydrosulfide (NaHS) attenuates the severity of I/R-induced liver injury [25,69]. These results indicate that endogenous H_2S may be important for alleviating hepatic I/R injury, and the elevated CSE/H_2S system exerts a compensatory role for H_2S production in the pathogenesis of hepatic IR injury. Whether the liver expression levels of CBS and 3-MST are altered in hepatic I/R injury remain to be studied in the future. Mechanistically, it is established that numerous signaling pathways mediate the protective roles of H_2S in hepatic I/R injury, including antioxidant and anti-apoptotic actions [67,70–72], inflammation [25,69,72], mitochondrial dysfunction [73], endoplasmic reticulum stress [74], autophagy [68,72,75,76], thioredoxin interacting protein (TXNIP) [77], and the nuclear factor erythroid2-related factor 2 (Nrf2) pathway [78]. However, it is noteworthy to mention that increased endogenous H_2S exacerbates the I/R-induced hepatic injury in rats with insulin resistant, and suppression of endogenous H_2S production may account for the protective effects of silymarin preconditioning against liver I/R injury in rats with insulin resistance [79]. These existing results suggest that H_2S confers a beneficial effect in ameliorating hepatic I/R injury, and targeting H_2S may provide a valuable way for the treatment of hepatic injury induced by I/R without insulin resistance. Simultaneously, the assessment of H_2S in hepatic I/R injury warrants caution under insulin resistance condition. In spite of this, the reparative hepatoprotection by H_2S might offer new therapeutic strategies for hepatic I/R injury in the absence of insulin resistance.

Role of H_2S in liver fibrosis

The fibrogenesis of chronic liver disease could disrupt the liver functional units and blood flow, leading to liver cirrhosis and even life-threatening clinical consequences [80,81]. With great efforts, significant advancements in the understanding of the underlying mechanisms of hepatic fibrosis and cirrhosis have grown exponentially. In the pathological process of hepatic fibrosis, it is well accepted that activated hepatic stellate cells (HSCs) is fundamental for the overproduction of extracellular matrix (ECM) in hepatic interstitium, thus resulting in hepatic fibrosis [82]. Current evidence suggests that inactivation of HSCs is an important mechanism for H_2S to prevent and treat liver fibrosis [83]. However, a recent report has illustrated that the H_2S production and CSE

expression are incremented during HSC activation, and exogenous H_2S promotes the proliferation of HSCs and evokes the fibrotic maker expressions of HSCs [84]. These contradictory results suggest that systemic treatment with H_2S in liver fibrosis should consider the cell-specific actions of H_2S. As a result, more experiments are required to determine the exact actions of H_2S on HSC activation and subsequent hepatic fibrosis.

The plasma H_2S levels are lower in rats with liver fibrosis, and intraperitoneal injection of H_2S synthase inhibitor propargylglycine (PPG) further promotes the fibrotic marker expression in the liver from liver cirrhosis group [85]. Both protein expressions of CSE and H_2S content tend to be inhibited in liver fibrosis model induced by carbon tetrachloride [86,87]. The evidence for the protective role of H_2S in liver fibrosis is supported by a finding that deficiency of CBS accelerates oxidative stress, inflammation, fibrosis in conjunction with steatosis in the liver [88]. In similarity, gene knockout of CSE triggers inflammatory response and exacerbates liver fibrosis by reducing H_2S production [86], indicating a potential role of the H_2S system in hepatic fibrosis. In addition, the cell-specific expressions and roles of H_2S-producing enzymes in hepatic fibrotic disease need to be fully understood.

Supplementation of NaHS, a donor of H_2S, protects liver function concomitant with an improvement of hepatic fibrosis and portal hypertension in mice treated with carbon tetrachloride [87]. The similar results are also observed in carbon tetrachloride-induced hepatic fibrosis rat model [89,90]. Diallyl trisulfide (DATS) is reported to reduce hepatic fibrosis in rats with fibrotic liver through elevation of the H_2S levels [91]. Moreover, S-allylcysteine (SAC), an endogenous donor of H_2S, attenuates liver fibrosis in carbon tetrachloride-induced rats through anti-oxidant, anti-inflammatory and anti-fibrotic effects [92]. The CSE/H_2S levels are inhibited in rats with hepatic fibrosis, this effect is reversed by caffeic acid phenethyl ester, thus exerting the effects of anti-hepatic fibrosis [93]. Exercise training markedly enhances H_2S contents and upregulates the hepatic expression levels of CBS, CSE and 3-MST in high fat diet (HFD)-fed mice, thereby contributing to its benefit on HFD-provoked hepatic fibrosis [94]. The present data suggest H_2S donors or restored bioavailability of H_2S have the potential for the treatment of liver fibrosis. Furthermore, long-lasting and safe H_2S-releasing donors can be developed to treat liver fibrosis in a proper way.

Mechanistically, the protective effects of H_2S against hepatic fibrosis might be attributed to suppression of oxidative stress and inflammation [87,90], inhibition of the signal transducer and activator of transcription 3 (STAT3)/Smad3 pathway [92], decreased phosphorylated p38 MAPK expression, and increased expression of phosphorylated Akt [95], as well as downregulation of angiotensin II type 1 receptor (AGTR1) [96]. It is revealed that the elevated carbon monoxide (CO) levels are observed in fibrotic liver, and inhibition of endogenous CO is believed to be a novel therapy for liver fibrosis [97,98]. Endogenous CO agonist cobalt protoporphyrin aggravates hepatic function and fibrosis via downregulated expressions of CSE and H_2S in the liver tissues [99]. By contrast, the increased expressions of CSE and H_2S by endogenous CO inhibitor zinc protoporphyrin IX are beneficial for liver function and fibrosis [99]. Collectively, these observations indicate that endogenous H_2S system or H_2S-releasing donors can be developed to treat liver fibrosis through various signaling pathways.

Role of H_2S in non-alcoholic fatty liver diseases (NAFLD)

Like the adipose tissue and intestinal tract, the liver is also an indispensable metabolic organ that governs lipid metabolism [100]. The liver is a critical location for the production and clearance of H_2S [16]. With the continuous advancement of gene editing technologies, the direct evidence for the relationship between H_2S and hepatic lipid metabolism can be achieved by using animals with gene deletion of CBS, CSE, and 3-MST, three H_2S-producing enzymes. CBS deficiency leads to the dysregulated expressions of gene involving in liver lipid homeostasis [101]. In animal models of CBS deficiency, the enhanced oxidative stress and hepatic lipid accumulation are observed [102]. Namekata et al. proposed that the abnormal metabolism in the liver from CBS knockout mice may be induced by hyperhomocysteinemia [102], which is a risk factor for hepatic steatosis [103]. Furthermore, the same group further demonstrated that the damaged β-oxidation of fatty acid and thiolase activity, and the aberrant levels of very low density lipoprotein (VLDL) are major contributing factors for hepatic steatosis in CBS knockout mice [102]. However, the H_2S levels in the liver from CBS knockout mice, and its relationship with hepatic lipid metabolism disruption was unclear in this study [102]. Notably, it was reported that hyperhomocysteinemia did not independently induce dyslipidemia in atherogenic diet-fed mice [104]. As a consequence, it is highly probable that hyperhomocysteinemia may not be a sole reason for abnormal liver lipid metabolism in mice with CBS deficiency. Because of the abnormal liver lipid metabolism in CBS knockout mice, additional experimental manipulations might meet difficulties when using such mice. To solve this problem, a new mouse model of CBS-deficient homocystinuria under the control of the human CBS promoter was created (designated HO) [105,106]. Like CBS knockout mice, HO mice exhibited the minimal CBS expression, but higher cystathionine levels [105,106]. Interestingly, the signs of liver lipid deposition and oxidative stress were not observed in these HO mice [105,106]. Aside from CBS, CSE is also a critical enzyme involving in hepatic H_2S production [16]. Although CSE knockout mice exhibit normal liver structure and functionality on normal chow diet feeding, hepatic lipid accumulation is aggravated in CSE deficient mice on the HFD condition [107–110]. In terms of 3-MST knockout mice, the phenotyping changes are much less than those of CBS deficiency mice, as 3-MST knockout mice showed no pathological features compared to their wild-type counterparts when fed by a normal diet [111]. However, partial deletion of 3-MST markedly improves hepatic steatosis in mice by HFD [26]. These above findings suggested that the H_2S-producing enzymes, CBS, CSE and 3-MST, are important regulators in hepatic lipid metabolism under physiological conditions. Specifically, CBS and CSE may inhibit, while 3-MST facilitates the disorders of liver lipid metabolism in response to HFD. Future studies are warranted to determine the precise roles of H_2S-producing enzymes in normal liver lipid homeostasis, such as β-oxidation of fatty acids, production and utilization of ketone bodies, cholesterol and triglyceride metabolism.

NAFLD is characterized by the presence of >5% steatosis in the presence of neither alcohol consumption or nor competing etiologies for hepatic steatosis [112]. NAFLD is defined as a disorder with excess fat in the liver, which is closely related with metabolic abnormalities, such as obesity, diabetes and dyslipidemia [113]. Epidemiological results have shown that NAFLD affects approximately 25% of the general adults around the world [114,115]. Unlike NAFLD, NASH is a complicated disease that is defined by steatosis, fibrosis, and necroinflammation under the NAFLD spectrum [116,117]. Similar to NAFLD, NASH could progress to liver cirrhosis and hepatocellular carcinoma (HCC), as well as liver failure [118,119]. A host of excellent reviews have highlighted the cellular and molecular mechanisms of NAFLD [120–123]. The critical contributors to NAFLD include lipid metabolism dysfunction, endoplasmic reticulum stress, oxidative stress, insulin resistance and inflammation. All of these pathological processes appear to be tightly linked with the H_2S system in the liver [107]. Despite of intensive investigations in the pathological mechanisms of NAFLD/NASH, so far, there are still unavailable therapies for

NAFLD/NASH. Therefore, it is pressing to identify new targets or strategies for the management of NAFLD/NASH.

Exogenous H_2S donors are reported to protect HepG2 hepatocytes against palmitic acid-induced inflammation, implying that the H_2S system may be a crucial target for the treatment of NAFLD through its anti-inflammatory effects [124]. The hepatic H_2S levels, CBS and CSE expressions are impaired in rats with NASH induced by a methionine/choline-deficient diet, and treatment with exogenous H_2S prevents the progression of NASH possibly through suppression of liver oxidative stress and inflammation [125]. Administration of NaHS, a H_2S donor, blocks the progression of NASH in mice fed with a methionine/choline-deficient diet [126]. The protective actions of H_2S are associated with suppression of chemokine CX3C motif receptor 1 (CX3CR1)-expressing inflammatory dendritic cells [126]. Likewise, S-propargyl-cysteine (SPRC), a new H_2S donor, protects the liver tissues from NASH mice induced by methionine/choline-deficient diet, and the protective effects of SPRC are associated with activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/Nrf2/heme oxygenase 1 (HO-1) signaling pathway [127]. In addition, exogenous application of H_2S mitigates the fatty liver via ameliorating the dysregulated lipid metabolism [128]. Treatment with NaHS markedly reduces hypertriglyceridemia and ameliorates NAFLD in HFD-fed mice via stimulating liver autophagic flux by amplifying the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway [129]. Interestingly, it has been shown that H_2S increases adipocyte numbers, but improves insulin resistance in HFD mice, suggesting that H_2S may confer a biphasic effect in fat tissues and liver tissues [130,131]. These results suggested that direct H_2S donors may grant a beneficial role in the treatment of fatty liver. Also, these observations provide the potential utilization of H_2S as a therapeutic molecule to ameliorate hepatic steatosis. Despite that H_2S stimulates the free fatty acid storage in fat tissues, it is widely accepted that H_2S could induce lipolysis in the liver and attenuates hepatic lipid deposition, thus triggering the protective effects against hepatic steatosis. According to the distinct roles of H_2S in different lipid metabolism organs, a better knowledge of the potential mechanisms by which H_2S regulates different metabolic pathway in the liver and other target organs might provide a more reasonable approach to treat hepatic steatosis. Furthermore, the dose, administration approaches and off-target effects should be considered when developing H_2S-based optimized therapeutic options for fatty liver.

Aside from the direct H_2S donors, some natural compounds have an impact on both hepatic lipid metabolism and H_2S production. Sulfated polysaccharide from Enteromorpha prolifera mimics the NaHS, a H_2S donor, to reduce the serum triglyceride level in HFD rats through upregulating hepatic mRNA and protein expressions of CBS, a main enzyme involving in the production of H_2S in the liver tissues [132]. G protein-coupled bile acid-activated receptor 1 agonist is demonstrated to reverse liver damage via stimulating H_2S generation in a mouse model of steatohepatitis [133]. Garlic oil has been shown to attenuate hepatic steatosis in mice treated with ethanol via regulation of fatty acid synthase (FAS) and mitochondrial dysfunction [134], and this agent could increase intracellular H_2S levels in human embryonic kidney cells in the presence of cysteine or glutathione [135]. Sulforaphane has the ability to ameliorate acute ethanol-induced fatty liver [136] and NAFLD in mice [137], and the protective effects of sulforaphane may be related with H_2S production [138]. In addition, exercise training obviously enhances the H_2S levels in the liver, thus attenuating systemic insulin resistance, glucose intolerance, hepatosteatosis and fibrosis in HFD-fed mice [94]. These findings indicated that induction of H_2S may be a promising therapeutic avenue for fatty liver. However, the underlying mechanisms by which such compounds and exercise promote endogenous H_2S production in the liver remains to be determined. It is worth pointing out that the possible side effects of these compounds warrant further research in addition to their beneficial effects on liver functions. Regardless of this, H_2S might be a therapeutic candidate against NAFLD/NASH. Novel H_2S donors and H_2S-releasing drugs may be beneficial for the treatment of NAFLD and NASH. However, the regulatory roles and mechanisms of H_2S in hepatic lipid accumulation, storage, and depletion are still largely lacking. Additional studies are warranted to address these questions during the development of NAFLD/NASH.

Role of H_2S in liver cancer

HCC is defined as the third cause of cancer-related death worldwide [139–141]. The management of HCC is still challenging due to the lack of timely and accurate diagnosis and treatment or evidence-based recommendations in this population [142]. HCC surveillance and early detection are recommended as effective approaches to increase the possibility of potentially curative treatment [143]. The majority of death in HCC patients is due to tumor recurrence [144]. As a consequence, identification of etiological mechanisms or novel strategies is urgently needed for the prevention and treatment of this prevalent malignancy [145].

In the liver system, a close relationship between H_2S and HCC has been demonstrated in recent years (Fig. 2) [49]. The H_2S-producing enzymes CSE and CBS expressions as well as H_2S levels are higher in human HCC cells, and suppression of the endogenous H_2S pathway obviously decreases the excessive growth of human HCC cells [146–148]. Furthermore, increased production of H_2S is associated with rapid proliferation of HCC cells in athymic mice [149]. Genetic deletion of CBS is capable of preventing the excessive proliferation of HCC cells [150]. A synthesized bioactive inhibitor of endogenous CBS substantially retards tumor growth in a xenograft mice model of liver cancer [150]. Moreover, blockade of the CBS/H2S system is vital for combination of curcumenol and laminarin to restrain the proliferation and metastasis of HCC cells [151]. Similarly, endogenous CSE/H_2S promotes human HCC cell proliferation via regulation of cell cycle progression, and activation of the PI3K/Akt pathway may result in the elevated CSE expression in HCC cell lines [28]. However, the precise molecular mechanism of CBS upregulation in HCC is unknown. Altogether, the activated endogenous H_2S system is fundamental for maintaining HCC carcinogenesis.

In agreement with the above results, exogenous NaHS (500 μM) treatment facilitates the growth of hepatoma cells, and this effect may be relied on the nuclear factor-kappa B (NF-κB) pathway [148]. In addition, the same group has also shown that exogenous H_2S (500 μM) facilitates the proliferation and migration of HCC cells by activating the STAT3/cyclooxygenase-2 (COX-2) signaling pathway [152]. However, treatment with NaHS (10^{-3}M) inhibits the migration and proliferation of HCC cells through attenuating the PI3K/Akt/mTOR pathway and promoting the induction of autophagy [153]. A H_2S donor GYY4137 (400 μM) inhibits the proliferation of human HCC cells via inactivation of the STAT3 pathway [154]. In a xenograft model with subcutaneous HepG2 cells, a large concentration of GYY4137 (50 mg/kg) effectively reduces tumor volume, whereas the low dose of GYY4137 (10 mg/kg) had no effect on tumor growth [154]. These findings imply that H_2S may act as a double-edged sword in the progression of human HCC. This notion is further confirmed by a finding that NaHS (10–100 μM) stimulates HCC cell proliferation and migration, whereas NaHS (600–1000 μM) exerts opposite effects [155]. The biphasic effects of NaHS are regulated by the epidermal growth factor receptor (EGFR)/extracellular regulated protein kinases (ERK)/matrix metalloproteinase 2 (MMP-2) and phosphatase and

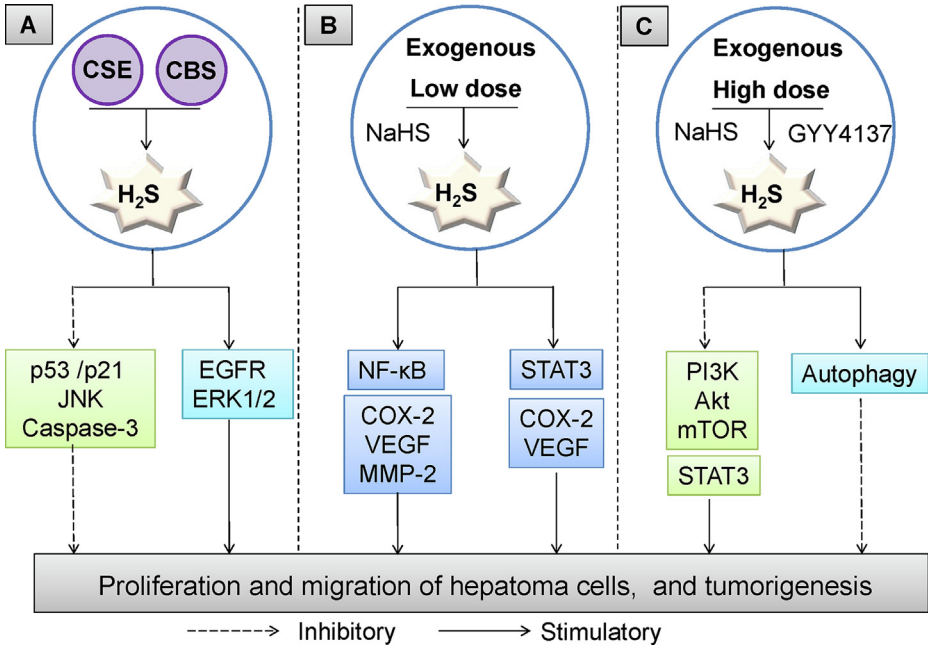

Fig. 2. Role of H_2S in HCC. (**A**) Over-activation of endogenous H_2S promotes the proliferation of liver cancer cells, this effect may be related with inhibition of p53, p21, JNK, caspase-3, PARP, Bax/Bcl-2 ratio, acceleration of cell cycle progression, and upregulation of EGFR, ERK1/2. (**B**) Treatment of hepatoma cells with NaHS (500 μM) stimulates the levels of CSE, CBS and activates NF-κB signaling, resulting in COX-2, VEGF and MMP-2 upregulations, and decreased caspase-3 activation, accompanied by increased HCC cell viability. In addition, activation of the STAT3/COX-2/VEGF axis is required for NaHS (500 μM) to stimulate HCC cell growth and migration. (**C**) Administration of NaHS (10^{-3} M) prevents the migration and proliferation of HCC cells via curbing the PI3K/Akt/mTOR signaling pathway and promoting the induction of autophagy. A H_2S donor GYY4137 (400 μM) diminishes tumor growth via inhibition of the STAT3 pathway.

tensin homolog deleted on chromosome ten (PTEN)/Akt signaling pathways [155]. On these grounds, a bell-shaped model may interpret the actions of the H_2S system on the pathogenesis of liver cancer. In other words, endogenous H_2S system or low concentrations of exogenous H_2S might trigger pro-cancer activities, while exogenous H_2S at higher concentrations may inhibit the progression of HCC. Therefore, pharmacological inhibition or genetic knockdown/knockout of the H_2S-generating enzymes and development of the H_2S-releasing donors (high dose) may be two distinct ways for liver cancer management.

Conclusions and future perspectives

This review summarizes and discusses the recent literatures about the roles and mechanisms of H_2S in several liver diseases, including NASH, hepatic fibrosis, hepatic I/R injury, and HCC. Deficiency of endogenous H_2S production is associated with NASH and hepatic fibrosis. It is still debatable for the roles of H_2S in hepatic I/R injury, suggesting that H_2S might serve as a double-edged sword in such liver disease. Thus, more research is warranted to address this discrepancy in the future. Additionally, endogenous H_2S production or lower exogenous H_2S may lead to liver cancer development, while exposure to H_2S with a high amount may exhibit anticancer properties. Thus, targeting the H_2S-producing enzymes may be a promising strategy for managing hepatic disorders.

Based on the published evidence, the important roles of H_2S in glycolipid metabolism, circadian rhythm, cell differentiation, and mitochondrial functions in the liver have been highlighted in recent years. However, one should bear in mind that the effects of endogenous H_2S, especially H_2S-producing enzyme 3-MST, on hepatic physiological processes are still in its infancy. It is believed that a comprehensive understanding of the exact roles and mechanisms of H_2S in liver health will largely advance new potential therapeutic applications of H_2S in preclinical and clinical research. Finally, the development of specific, sensitive and biologically compatible H_2S probes and novel long-lasting H_2S donors will certainly provide unique opportunities for the management of hepatic disorders in the near future.

Compliance with ethics requirements

This review article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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References

[1] Mishanina TV, Libiad M, Banerjee R. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. Nat Chem Biol 2015;11:457–64.
[2] Liu YH, Lu M, Hu LF, Wong PT, Webb GD, Bian JS. Hydrogen sulfide in the mammalian cardiovascular system. Antioxid Redox Signal 2012;17:141–85.
[3] Nagpure BV, Bian JS. Interaction of hydrogen sulfide with nitric oxide in the cardiovascular system. Oxid Med Cell Longev 2016;2016:6904327.
[4] Hu LF, Lu M, Hon Wong PT, Bian JS. Hydrogen sulfide: neurophysiology and neuropathology. Antioxid Redox Signal 2011;15:405–19.
[5] Nagpure BV, Bian JS. Brain, learning, and memory: role of H2S in neurodegenerative diseases. Handb Exp Pharmacol 2015;230:193–215.
[6] Gong QH, Shi XR, Hong ZY, Pan LL, Liu XH, Zhu YZ. A new hope for neurodegeneration: possible role of hydrogen sulfide. J Alzheimers Dis 2011;24(Suppl 2):173–82.
[7] Gambari L, Grigolo B, Grassi F. Hydrogen sulfide in bone tissue regeneration and repair: state of the art and new perspectives. Int J Mol Sci 2019;20. pii: E5231.
[8] Sun HJ, Wu ZY, Cao L, Zhu MY, Liu TT, Guo L, et al. Hydrogen sulfide: recent progression and perspectives for the treatment of diabetic nephropathy. Molecules 2019;24. pii: E2857.

[9] Cao X, Ding L, Xie ZZ, Yang Y, Whiteman M, Moore PK, et al. A Review of hydrogen sulfide synthesis, metabolism, and measurement: is modulation of hydrogen sulfide a novel therapeutic for cancer?. Antioxid Redox Signal 2019;31:1–38.
[10] Untereiner A, Wu L. Hydrogen sulfide and glucose homeostasis: a tale of sweet and the stink. Antioxid Redox Signal 2018;28:1463–82.
[11] Das UN. Ageing: Is there a role for arachidonic acid and other bioactive lipids? A review. J Adv Res 2018;11:67–79.
[12] Olas B. Hydrogen sulfide in signaling pathways. Clin Chim Acta 2015;439:212–8.
[13] Zhang S, Pan C, Zhou F, Yuan Z, Wang H, Cui W, et al. Hydrogen sulfide as a potential therapeutic target in fibrosis. Oxid Med Cell Longev 2015;2015:593407.
[14] Searcy DG, Lee SH. Sulfur reduction by human erythrocytes. J Exp Zool 1998;282:310–22.
[15] Wang R. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter?. FASEB J 2002;16:1792–8.
[16] Mani S, Cao W, Wu L, Wang R. Hydrogen sulfide and the liver. Nitric Oxide 2014;41:62–71.
[17] Gao B, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. Hepatology 2008;47:729–36.
[18] Shi D, Chen J, Wang J, Yao J, Huang Y, Zhang G, et al. Circadian clock genes in the metabolism of non-alcoholic fatty liver disease. Front Physiol 2019;10:423.
[19] Chen K, Zhong J, Hu L, Li R, Du Q, Cai J, et al. The role of xenobiotic receptors on hepatic glycolipid metabolism. Curr Drug Metab 2019;20:29–35.
[20] Andrade RJ, Chalasani N, Bjornsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, et al. Drug-induced liver injury. Nat Rev Dis Primers 2019;5:58.
[21] Norris EJ, Culberson CR, Narasimhan S, Clemens MG. The liver as a central regulator of hydrogen sulfide. Shock 2011;36:242–50.
[22] Ju Y, Untereiner A, Wu L, Yang G. H2S-induced S-sulfhydration of pyruvate carboxylase contributes to gluconeogenesis in liver cells. Biochim Biophys Acta 2015;1850:2293–303.
[23] Pichette J, Gagnon J. Implications of hydrogen sulfide in glucose regulation: how H2S can alter glucose homeostasis through metabolic hormones. Oxid Med Cell Longev 2016;2016:3285074.
[24] Untereiner AA, Fu M, Modis K, Wang R, Ju Y, Wu L. Stimulatory effect of CSE-generated H2S on hepatic mitochondrial biogenesis and the underlying mechanisms. Nitric Oxide 2016;58:67–76.
[25] Kang K, Zhao M, Jiang H, Tan G, Pan S, Sun X. Role of hydrogen sulfide in hepatic ischemia-reperfusion-induced injury in rats. Liver Transpl 2009;15:1306–14.
[26] Li M, Xu C, Shi J, Ding J, Wan X, Chen D, et al. Fatty acids promote fatty liver disease via the dysregulation of 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. Gut 2018;67:2169–80.
[27] Song K, Li Q, Yin XY, Lu Y, Liu CF, Hu LF. Hydrogen sulfide: a therapeutic candidate for fibrotic disease?. Oxid Med Cell Longev 2015;2015:458720.
[28] Yin P, Zhao C, Li Z, Mei C, Yao W, Liu Y, et al. Sp1 is involved in regulation of cystathionine gamma-lyase gene expression and biological function by PI3K/Akt pathway in human hepatocellular carcinoma cell lines. Cell Signal 2012;24:1229–40.
[29] Li L, Rose P, Moore PK. Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol 2011;51:169–87.
[30] Kimura H. The physiological role of hydrogen sulfide and beyond. Nitric Oxide 2014;41:4–10.
[31] Polhemus DJ, Lefer DJ. Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. Circ Res 2014;114:730–7.
[32] Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, et al. H2S signals through protein S-sulfhydration. Sci Signal 2009;2:ra72.
[33] Fu M, Zhang W, Wu L, Yang G, Li H, Wang R. Hydrogen sulfide (H2S) metabolism in mitochondria and its regulatory role in energy production. Proc Natl Acad Sci U S A 2012;109:2943–8.
[34] Shibuya N, Koike S, Tanaka M, Ishigami-Yuasa M, Kimura Y, Ogasawara Y, et al. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. Nat Commun 2013;4:1366.
[35] Nagahara N, Ito T, Kitamura H, Nishino T. Tissue and subcellular distribution of mercaptopyruvate sulfurtransferase in the rat: confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis. Histochem Cell Biol 1998;110:243–50.
[36] Kimura H. Production and physiological effects of hydrogen sulfide. Antioxid Redox Signal 2014;20:783–93.
[37] Nin DS, Binte Idres S, Song Z, Moore PK, Deng LW. Biological effects of GYY4137 and other phosphorothioate-based hydrogen sulfide donors. Antioxid Redox Signal 2020;32:145–58.
[38] Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, et al. Hydrogen sulfide mediates the vasoactivity of garlic. Proc Natl Acad Sci U S A 2007;104:17977–82.
[39] Kolluru GK, Shen X, Bir SC, Kevil CG. Hydrogen sulfide chemical biology: pathophysiological roles and detection. Nitric Oxide 2013;35:5–20.
[40] Olson KR, Deleon ER, Gao Y, Hurley K, Sadauskas V, Batz C, et al. Thiosulfate: a readily accessible source of hydrogen sulfide in oxygen sensing. Am J Physiol Regul Integr Comp Physiol 2013;305:R592–603.
[41] Predmore BL, Kondo K, Bhushan S, Zlatopolsky MA, King AL, Aragon JP, et al. The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability. Am J Physiol Heart Circ Physiol 2012;302:H2410–8.
[42] Snijder PM, Frenay AR, de Boer RA, Pasch A, Hillebrands JL, Leuvenink HG, et al. Exogenous administration of thiosulfate, a donor of hydrogen sulfide, attenuates angiotensin II-induced hypertensive heart disease in rats. Br J Pharmacol 2015;172:1494–504.
[43] Imai T, Kosuge Y, Endo-Umeda K, Miyagishi H, Ishige K, Makishima M, et al. Protective effect of S-allyl-L-cysteine against endoplasmic reticulum stress-induced neuronal death is mediated by inhibition of calpain. Amino Acids 2014;46:385–93.
[44] Ahmad A, Gero D, Olah G, Szabo C. Effect of endotoxemia in mice genetically deficient in cystathionine-gamma-lyase, cystathionine-beta-synthase or 3-mercaptopyruvate sulfurtransferase. Int J Mol Med 2016;38:1683–92.
[45] Kabil O, Vitvitsky V, Xie P, Banerjee R. The quantitative significance of the transsulfuration enzymes for H2S production in murine tissues. Antioxid Redox Signal 2011;15:363–72.
[46] Manna P, Gungor N, McVie R, Jain SK. Decreased cystathionine-gamma-lyase (CSE) activity in livers of type 1 diabetic rats and peripheral blood mononuclear cells (PBMC) of type 1 diabetic patients. J Biol Chem 2014;289:11767–78.
[47] Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, Rizzo G, et al. The third gas: H2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. Hepatology 2005;42:539–48.
[48] Zhang L, Yang G, Untereiner A, Ju Y, Wu L, Wang R. Hydrogen sulfide impairs glucose utilization and increases gluconeogenesis in hepatocytes. Endocrinology 2013;154:114–26.
[49] Wu DD, Wang DY, Li HM, Guo JC, Duan SF. Hydrogen sulfide as a novel regulatory factor in liver health and disease. Oxid Med Cell Longev 2019;2019:3831713.
[50] Altamirano-Barrera A, Uribe M, Chavez-Tapia NC, Nuno-Lambarri N. The role of the gut microbiota in the pathology and prevention of liver disease. J Nutr Biochem 2018;60:1–8.
[51] Beltowski J, Wojcicka G, Jamroz-Wisniewska A. Hydrogen sulfide in the regulation of insulin secretion and insulin sensitivity: Implications for the pathogenesis and treatment of diabetes mellitus. Biochem Pharmacol 2018;149:60–76.
[52] Carter RN, Morton NM. Cysteine and hydrogen sulphide in the regulation of metabolism: insights from genetics and pharmacology. J Pathol 2016;238:321–32.
[53] Chan MV, Wallace JL. Hydrogen sulfide-based therapeutics and gastrointestinal diseases: translating physiology to treatments. Am J Physiol Gastrointest Liver Physiol 2013;305:G467–73.
[54] Cipriani S, Mencarelli A. Hydrogen sulfide in gastrointestinal and liver physiopathology. Inflamm Allergy Drug Targets 2011;10:92–102.
[55] Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 2012;92:791–896.
[56] Gu X, Zhu YZ. Therapeutic applications of organosulfur compounds as novel hydrogen sulfide donors and/or mediators. Expert Rev Clin Pharmacol 2011;4:123–33.
[57] Fiorucci S, Distrutti E, Cirino G, Wallace JL. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. Gastroenterology 2006;131:259–71.
[58] Iwakiri Y, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. Hepatology 2006;43:S121–31.
[59] Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nat Med 2011;17:1391–401.
[60] Wu D, Wang J, Li H, Xue M, Ji A, Li Y. Role of Hydrogen Sulfide in Ischemia-Reperfusion Injury. Oxid Med Cell Longev 2015;2015:186908.
[61] Nastos C, Kalimeris K, Papoutsidakis N. Global consequences of liver ischemia/reperfusion injury. Oxid Med Cell Longev 2014;2014:906965.
[62] Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation–from bench to bedside. Nat Rev Gastroenterol Hepatol 2013;10:79–89.
[63] Klune JR, Tsung A. Molecular biology of liver ischemia/reperfusion injury: established mechanisms and recent advancements. Surg Clin North Am 2010;90:665–77.
[64] Mukhopadhyay P, Horvath B, Zsengeller Z, Batkai S, Cao Z, Kechrid M, et al. Mitochondrial reactive oxygen species generation triggers inflammatory response and tissue injury associated with hepatic ischemia-reperfusion: therapeutic potential of mitochondrially targeted antioxidants. Free Radic Biol Med 2012;53:1123–38.
[65] Fan C, Zwacka RM, Engelhardt JF. Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med (Berl) 1999;77:577–92.
[66] Lowicka E, Beltowski J. Hydrogen sulfide (H2S) – the third gas of interest for pharmacologists. Pharmacol Rep 2007;59:4–24.
[67] Jha S, Calvert JW, Duranski MR, Ramachandran A, Lefer DJ. Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. Am J Physiol Heart Circ Physiol 2008;295:H801–6.
[68] Lu M, Jiang X, Tong L, Zhang F, Ma L, Dong X, et al. MicroRNA-21-regulated activation of the Akt pathway participates in the protective effects of H2S against liver ischemia-reperfusion injury. Biol Pharm Bull 2018;41:229–38.
[69] Kang K, Jiang HC, Zhao MY, Sun XY, Pan SH. Protection of CSE/H2S system in hepatic ischemia reperfusion injury in rats. Zhonghua Wai Ke Za Zhi 2010;48:924–8.
[70] Shaik IH, George JM, Thekkumkara TJ, Mehvar R. Protective effects of diallyl sulfide, a garlic constituent, on the warm hepatic ischemia-reperfusion injury in a rat model. Pharm Res 2008;25:2231–42.

[71] Haga S, Remington SJ, Morita N, Terui K, Ozaki M. Hepatic ischemia induced immediate oxidative stress after reperfusion and determined the severity of the reperfusion-induced damage. Antioxid Redox Signal 2009;11:2563–72.
[72] Cheng P, Wang F, Chen K, Shen M, Dai W, Xu L, et al. Hydrogen sulfide ameliorates ischemia/reperfusion-induced hepatitis by inhibiting apoptosis and autophagy pathways. Mediators Inflamm 2014;2014:935251.
[73] Liu Y, Kalogeris T, Wang M, Zuidema MY, Wang Q, Dai H, et al. Hydrogen sulfide preconditioning or neutrophil depletion attenuates ischemia-reperfusion-induced mitochondrial dysfunction in rat small intestine. Am J Physiol Gastrointest Liver Physiol 2012;302:G44–54.
[74] Ruan Z, Liang M, Deng X, Lai M, Shang L, Su X. Exogenous hydrogen sulfide protects fatty liver against ischemia-reperfusion injury by regulating endoplasmic reticulum stress-induced autophagy in macrophage through mediating the class A scavenger receptor pathway in rats. Cell Biol Int 2019. doi: https://doi.org/10.1002/cbin.11234.
[75] Wang D, Ma Y, Li Z, Kang K, Sun X, Pan S, et al. The role of AKT1 and autophagy in the protective effect of hydrogen sulphide against hepatic ischemia/reperfusion injury in mice. Autophagy 2012;8:954–62.
[76] Zhang Q, Fu H, Zhang H, Xu F, Zou Z, Liu M, et al. Hydrogen sulfide preconditioning protects rat liver against ischemia/reperfusion injury by activating Akt-GSK-3beta signaling and inhibiting mitochondrial permeability transition. PLoS ONE 2013;8:e74422.
[77] Du J, Wang Q, Li QM, Zhang BM, Xie KL, Wang GL. Alternation of thioredoxin system in postconditioning with hydrogen sulfide against hepatic ischemia-reperfusion injury in rats. Zhonghua Yi Xue Za Zhi 2012;92:2607–10.
[78] Huang X, Gao Y, Qin J, Lu S. The role of miR-34a in the hepatoprotective effect of hydrogen sulfide on ischemia/reperfusion injury in young and old rats. PLoS ONE 2014;9:e113305.
[79] Younis NN, Shaheen MA, Mahmoud MF. Silymarin preconditioning protected insulin resistant rats from liver ischemia-reperfusion injury: role of endogenous H2S. J Surg Res 2016;204:398–409.
[80] Pinzani M, Macias-Barragan J. Update on the pathophysiology of liver fibrosis. Expert Rev Gastroenterol Hepatol 2010;4:459–72.
[81] Bosetti C, Levi F, Lucchini F, Zatonski WA, Negri E, La Vecchia C. Worldwide mortality from cirrhosis: an update to 2002. J Hepatol 2007;46:827–39.
[82] Ni MM, Wang YR, Wu WW, Xia CC, Zhang YH, Xu J, et al. Novel insights on notch signaling pathways in liver fibrosis. Eur J Pharmacol 2018;826:66–74.
[83] Zhang F, Jin H, Wu L, Shao J, Zhu X, Chen A, et al. Diallyl trisulfide suppresses oxidative stress-induced activation of hepatic stellate cells through production of hydrogen sulfide. Oxid Med Cell Longev 2017;2017:1406726.
[84] Damba T, Zhang M, Buist-Homan M, van Goor H, Faber KN, Moshage H. Hydrogen sulfide stimulates activation of hepatic stellate cells through increased cellular bio-energetics. Nitric Oxide 2019;92:26–33.
[85] Wei W, Wang C, Li D. The content of hydrogen sulfide in plasma of cirrhosis rats combined with portal hypertension and the correlation with indexes of liver function and liver fibrosis. Exp Ther Med 2017;14:5022–6.
[86] Ci L, Yang X, Gu X, Li Q, Guo Y, Zhou Z, et al. Cystathionine gamma-lyase deficiency exacerbates CCl4-induced acute hepatitis and fibrosis in the mouse liver. Antioxid Redox Signal 2017;27:133–49.
[87] Tan G, Pan S, Li J, Dong X, Kang K, Zhao M, et al. Hydrogen sulfide attenuates carbon tetrachloride-induced hepatotoxicity, liver cirrhosis and portal hypertension in rats. PLoS ONE 2011;6:e25943.
[88] Robert K, Nehme J, Bourdon E, Pivert G, Friguet B, Delcayre C, et al. Cystathionine beta synthase deficiency promotes oxidative stress, fibrosis, and steatosis in mice liver. Gastroenterology 2005;128:1405–15.
[89] Shen Q, Qin Z, Lu A. Preventive effect of exogenous hydrogen sulfide on hepatic fibrosis in rats. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2012;37:911–5.
[90] Fan HN, Wang HJ, Yang-Dan CR, Ren L, Wang C, Li YF, et al. Protective effects of hydrogen sulfide on oxidative stress and fibrosis in hepatic stellate cells. Mol Med Rep 2013;7:247–53.
[91] Zhu X, Zhang F, Zhou L, Kong D, Chen L, Lu Y, et al. Diallyl trisulfide attenuates carbon tetrachloride-caused liver injury and fibrogenesis and reduces hepatic oxidative stress in rats. Naunyn Schmiedebergs Arch Pharmacol 2014;387:445–55.
[92] Gong Z, Ye H, Huo Y, Wang L, Huang Y, Huang M, et al. S-allyl-cysteine attenuates carbon tetrachloride-induced liver fibrosis in rats by targeting STAT3/SMAD3 pathway. Am J Transl Res 2018;10:1337–46.
[93] Shi Y, Guo L, Shi L, Yu J, Song M, Li Y. Caffeic acid phenethyl ester inhibit hepatic fibrosis by nitric oxide synthase and cystathionine gamma-lyase in rats. Med Sci Monit 2015;21:2774–80.
[94] Wang B, Zeng J, Gu Q. Exercise restores bioavailability of hydrogen sulfide and promotes autophagy influx in livers of mice fed with high-fat diet. Can J Physiol Pharmacol 2017;95:667–74.
[95] Fan HN, Wang HJ, Ren L, Ren B, Dan CR, Li YF, et al. Decreased expression of p38 MAPK mediates protective effects of hydrogen sulfide on hepatic fibrosis. Eur Rev Med Pharmacol Sci 2013;17:644–52.
[96] Fan HN, Chen NW, Shen WL, Zhao XY, Zhang J. Endogenous hydrogen sulfide is associated with angiotensin II type 1 receptor in a rat model of carbon tetrachloride-induced hepatic fibrosis. Mol Med Rep 2015;12:3351–8.
[97] Wang QM, Duan ZJ, Du JL, Guo SB, Sun XY, Liu Z. Heme oxygenase/carbon monoxide pathway inhibition plays a role in ameliorating fibrosis following splenectomy. Int J Mol Med 2013;31:1186–94.
[98] Wang QM, Du JL, Duan ZJ, Guo SB, Sun XY, Liu Z. Inhibiting heme oxygenase-1 attenuates rat liver fibrosis by removing iron accumulation. World J Gastroenterol 2013;19:2921–34.
[99] Guo SB, Duan ZJ, Wang QM, Zhou Q, Li Q, Sun XY. Endogenous carbon monoxide downregulates hepatic cystathionine-gamma-lyase in rats with liver cirrhosis. Exp Ther Med 2015;10:2039–46.
[100] Nguyen P, Leray V, Diez M, Serisier S, Le Bloc'h J, Siliart B, et al. Liver lipid metabolism. J Anim Physiol Anim Nutr (Berl) 2008;92:272–83.
[101] Hamelet J, Demuth K, Paul JL, Delabar JM, Janel N. Hyperhomocysteinemia due to cystathionine beta synthase deficiency induces dysregulation of genes involved in hepatic lipid homeostasis in mice. J Hepatol 2007;46:151–9.
[102] Namekata K, Enokido Y, Ishii I, Nagai Y, Harada T, Kimura H. Abnormal lipid metabolism in cystathionine beta-synthase-deficient mice, an animal model for hyperhomocysteinemia. J Biol Chem 2004;279:52961–9.
[103] Yao L, Cao B, Cheng Q, Cai W, Ye C, Liang J, et al. Inhibition of soluble epoxide hydrolase ameliorates hyperhomocysteinemia-induced hepatic steatosis by enhancing beta-oxidation of fatty acid in mice. Am J Physiol Gastrointest Liver Physiol 2019;316:G527–38.
[104] Zhou J, Moller J, Danielsen CC, Bentzon J, Ravn HB, Austin RC, et al. Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2001;21:1470–6.
[105] Maclean KN, Sikora J, Kozich V, Jiang H, Greiner LS, Kraus E, et al. A novel transgenic mouse model of CBS-deficient homocystinuria does not incur hepatic steatosis or fibrosis and exhibits a hypercoagulative phenotype that is ameliorated by betaine treatment. Mol Genet Metab 2010;101:153–62.
[106] Maclean KN, Greiner LS, Evans JR, Sood SK, Lhotak S, Markham NE, et al. Cystathionine protects against endoplasmic reticulum stress-induced lipid accumulation, tissue injury, and apoptotic cell death. J Biol Chem 2012;287:31994–2005.
[107] Sarna LK, Siow YL, O K. The CBS/CSE system: a potential therapeutic target in NAFLD?. Can J Physiol Pharmacol 2015;93(1):1–11.
[108] Mani S, Yang G, Wang R. A critical life-supporting role for cystathionine gamma-lyase in the absence of dietary cysteine supply. Free Radic Biol Med 2011;50:1280–7.
[109] Mani S, Li H, Untereiner A, Wu L, Yang G, Austin RC, et al. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. Circulation 2013;127:2523–34.
[110] Loiselle JJ, Yang G, Wu L. Hydrogen sulfide and hepatic lipid metabolism - a critical pairing for liver health. Br J Pharmacol 2020;177:757–68.
[111] Nagahara N, Nagano M, Ito T, Shimamura K, Akimoto T, Suzuki H. Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase-knockout mice exhibit increased anxiety-like behaviors: a model for human mercaptolactate-cysteine disulfiduria. Sci Rep 2013;3:1986.
[112] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Am J Gastroenterol 2012;107:811–26.
[113] Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2:901–10.
[114] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84.
[115] Konerman MA, Jones JC, Harrison SA. Pharmacotherapy for NASH: Current and emerging. J Hepatol 2018;68:362–75.
[116] Zhou JH, Cai JJ, She ZG, Li HL. Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice. World J Gastroenterol 2019;25:1307–26.
[117] Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156. 1264–81.e4.
[118] Kennedy-Martin T, Bae JP, Paczkowski R, Freeman E. Health-related quality of life burden of nonalcoholic steatohepatitis: a robust pragmatic literature review. J Patient Rep Outcomes 2017;2:28.
[119] Chow MD, Lee YH, Guo GL. The role of bile acids in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Mol Aspects Med 2017;56:34–44.
[120] Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. Science 2011;332:1519–23.
[121] Matherly SC, Puri P. Mechanisms of simple hepatic steatosis: not so simple after all. Clin Liver Dis 2012;16:505–24.
[122] Harmon RC, Tiniakos DG, Argo CK. Inflammation in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol 2011;5:189–200.
[123] Polyzos SA, Kountouras J, Zavos C, Deretzi G. Nonalcoholic fatty liver disease: multimodal treatment options for a pathogenetically multiple-hit disease. J Clin Gastroenterol 2012;46:272–84.
[124] Wang YD, Li JY, Qin Y, Liu Q, Liao ZZ, Xiao XH. Exogenous hydrogen sulfide alleviates-induced intracellular inflammation in HepG2 cells. Exp Clin Endocrinol Diabetes 2020;128:137–43.
[125] Luo ZL, Tang LJ, Wang T, Dai RW, Ren JD, Cheng L, et al. Effects of treatment with hydrogen sulfide on methionine-choline deficient diet-induced non-alcoholic steatohepatitis in rats. J Gastroenterol Hepatol 2014;29:215–22.
[126] Sutti S, Locatelli I, Bruzzi S, Jindal A, Vacchiano M, Bozzola C, et al. CX3CR1-expressing inflammatory dendritic cells contribute to the progression of steatohepatitis. Clin Sci (Lond) 2015;129:797–808.
[127] Li W, Ma F, Zhang L, Huang Y, Li X, Zhang A, et al. S-propargyl-cysteine exerts a novel protective effect on methionine and choline deficient diet-induced

fatty liver via Akt/Nrf2/HO-1 pathway. Oxid Med Cell Longev 2016;2016:4690857.
[128] Wu D, Zheng N, Qi K, Cheng H, Sun Z, Gao B, et al. Exogenous hydrogen sulfide mitigates the fatty liver in obese mice through improving lipid metabolism and antioxidant potential. Med Gas Res 2015;5:1.
[129] Sun L, Zhang S, Yu C, Pan Z, Liu Y, Zhao J, et al. Hydrogen sulfide reduces serum triglyceride by activating liver autophagy via the AMPK-mTOR pathway. Am J Physiol Endocrinol Metab 2015;309:E925–35.
[130] Cai J, Shi X, Wang H, Fan J, Feng Y, Lin X, et al. Cystathionine gamma lyase-hydrogen sulfide increases peroxisome proliferator-activated receptor gamma activity by sulfhydration at C139 site thereby promoting glucose uptake and lipid storage in adipocytes. Biochim Biophys Acta 2016;1861:419–29.
[131] Yang G, Ju Y, Fu M, Zhang Y, Pei Y, Racine M, et al. Cystathionine gamma-lyase/hydrogen sulfide system is essential for adipogenesis and fat mass accumulation in mice. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863:165–76.
[132] Ren R, Yang Z, Zhao A, Huang Y, Lin S, Gong J, et al. Sulfated polysaccharide from Enteromorpha prolifera increases hydrogen sulfide production and attenuates non-alcoholic fatty liver disease in high-fat diet rats. Food Funct 2018;9:4376–83.
[133] Carino A, Marchiano S, Biagioli M, Bucci M, Vellecco V, Brancaleone V, et al. Agonism for the bile acid receptor GPBAR1 reverses liver and vascular damage in a mouse model of steatohepatitis. FASEB J 2019;33:2809–22.
[134] Zeng T, Zhang CL, Song FY, Zhao XL, Xie KQ. Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR-alpha, and CYP2E1. Food Chem Toxicol 2012;50:485–91.
[135] DeLeon ER, Gao Y, Huang E, Olson KR. Garlic oil polysulfides: H2S- and O2-independent prooxidants in buffer and antioxidants in cells. Am J Physiol Regul Integr Comp Physiol 2016;310:R1212–25.
[136] Zhou R, Lin J, Wu D. Sulforaphane induces Nrf2 and protects against CYP2E1-dependent binge alcohol-induced liver steatosis. Biochim Biophys Acta 2014;1840:209–18.
[137] Yang G, Lee HE, Lee JY. A pharmacological inhibitor of NLRP3 inflammasome prevents non-alcoholic fatty liver disease in a mouse model induced by high fat diet. Sci Rep 2016;6:24399.
[138] Pei Y, Wu B, Cao Q, Wu L, Yang G. Hydrogen sulfide mediates the anti-survival effect of sulforaphane on human prostate cancer cells. Toxicol Appl Pharmacol 2011;257:420–8.
[139] Zhu RX, Seto WK, Lai CL, Yuen MF. Epidemiology of hepatocellular carcinoma in the Asia-Pacific Region. Gut Liver 2016;10:332–9.
[140] Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: possible targets and future directions. Nat Rev Clin Oncol 2011;8:292–301.
[141] Shi X, Zhu HR, Liu TT, Shen XZ, Zhu JM. The Hippo pathway in hepatocellular carcinoma: Non-coding RNAs in action. Cancer Lett 2017;400:175–82.
[142] Arora SP, Liposits G, Caird S, Dunne RF, Moffat GT, Okonji D, et al. Hepatocellular carcinoma in older adults: A comprehensive review by Young International Society of Geriatric Oncology. J Geriatr Oncol 2020;11:557–65.
[143] Yang JD, Hainaut P, Gores GJ. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019;16:589–604.
[144] Shi JH, Line PD. Effect of liver regeneration on malignant hepatic tumors. World J Gastroenterol 2014;20:16167–77.
[145] Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2015;12:681–700.
[146] Pan Y, Ye S, Yuan D, Zhang J, Bai Y, Shao C. Hydrogen sulfide (H2S)/cystathionine gamma-lyase (CSE) pathway contributes to the proliferation of hepatoma cells. Mutat Res 2014;763–764:10–8.
[147] Jia H, Ye J, You J, Shi X, Kang W, Wang T. Role of the cystathionine beta-synthase/H2S system in liver cancer cells and the inhibitory effect of quinolone-indolone conjugate QIC2 on the system. Oncol Rep 2017;37:3001–9.
[148] Zhen Y, Pan W, Hu F, Wu H, Feng J, Zhang Y, et al. Exogenous hydrogen sulfide exerts proliferation/anti-apoptosis/angiogenesis/migration effects via amplifying the activation of NF-kappaB pathway in PLC/PRF/5 hepatoma cells. Int J Oncol 2015;46:2194–204.
[149] Sanokawa-Akakura R, Ostrakhovitch EA, Akakura S, Goodwin S, Tabibzadeh S. A H2S-Nampt dependent energetic circuit is critical to survival and cytoprotection from damage in cancer cells. PLoS ONE 2014;9:e108537.
[150] Wang L, Cai H, Hu Y, Liu F, Huang S, Zhou Y, et al. A pharmacological probe identifies cystathionine beta-synthase as a new negative regulator for ferroptosis. Cell Death Dis 2018;9:1005.
[151] Han H, Wang L, Liu Y, Shi X, Zhang X, Li M, et al. Combination of curcuma zedoary and kelp inhibits growth and metastasis of liver cancer in vivo and in vitro via reducing endogenous H2S levels. Food Funct 2019;10:224–34.
[152] Zhen Y, Wu Q, Ding Y, Zhang W, Zhai Y, Lin X, et al. Exogenous hydrogen sulfide promotes hepatocellular carcinoma cell growth by activating the STAT3-COX-2 signaling pathway. Oncol Lett 2018;15:6562–70.
[153] Wang SS, Chen YH, Chen N, Wang LJ, Chen DX, Weng HL, et al. Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway. Cell Death Dis 2017;8:e2688.
[154] Lu S, Gao Y, Huang X, Wang X. GYY4137, a hydrogen sulfide (H(2)S) donor, shows potent anti-hepatocellular carcinoma activity through blocking the STAT3 pathway. Int J Oncol 2014;44:1259–67.
[155] Wu D, Li M, Tian W, Wang S, Cui L, Li H, et al. Hydrogen sulfide acts as a double-edged sword in human hepatocellular carcinoma cells through EGFR/ERK/MMP-2 and PTEN/AKT signaling pathways. Sci Rep 2017;7:5134.

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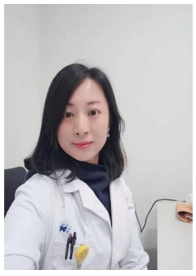

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