Hydrogen Sulfide and Its Donors: Keys to Unlock the Chains of Nonalcoholic Fatty Liver Disease

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Abstract: Hydrogen sulfide (H₂S) has emerged as the third “gasotransmitters” and has a crucial function in the diversity of physiological functions in mammals. In particular, H₂S is considered indispensable in preventing the development of liver inflammation in the case of excessive caloric ingestion. Note that the concentration of endogenous H₂S was usually low, making it difficult to discern the precise biological functions. Therefore, exogenous delivery of H₂S is conducive to probe the physiological and pathological roles of this gas in cellular and animal studies. In this review, the production and metabolic pathways of H₂S in vivo, the types of donors currently used for H₂S release, and study evidence of H₂S improvement effects on nonalcoholic fatty liver disease are systematically introduced.

Keywords: hydrogen sulfide; non-alcoholic fatty liver disease; H₂S donor

1. Introduction

Hydrogen sulfide (H₂S), well-known for its “rotten egg” odor, has been thoroughly described as a deadly toxic gas for centuries [1,2]. However, H₂S has also been recognized as the third gaseous signaling molecule besides carbon monoxide (CO) and nitric oxide (NO) [3,4], improving various pathological processes, including angiogenesis, neuromodulation, inflammation, apoptosis, and tumorigenesis. Paradoxically, H₂S, on one hand, acts as a physiological intercellular messenger to improve the therapeutic effect in some diseases; on the other side, it shows cytotoxic activity at high concentrations above physiological levels. Since the toxic effect of H₂S is beyond its physiological range as a gas transmitter, it will not be discussed in this article.

The liver is a vital organ in the production and metabolism of H₂S. In the last decade, accumulating evidence has suggested the critical function of H₂S in the occurrence and development of several liver diseases, such as drug-induced liver injury caused by acetaminophen, acute liver injury, ischemia-reperfusion, and liver cirrhosis [5–9]. Furthermore, numerous studies revealed that the endogenous production of H₂S was impaired in high-fat diet (HFD)-fed mice with non-alcoholic-steatohepatitis (NASH).

Nonalcoholic fatty liver disease (NAFLD) is characterized by abnormal lipid accumulation in the liver of individuals who do not drink alcohol excessively [5]. NAFLD is a common form of chronic liver disease [6] that consists of four major stages: nonalcoholic fatty liver (NAFL), NASH, hepatic fibrosis (HF), and hepatic cirrhosis (HC) [7,8]. NAFL can lead to hepatic failure, which is characterized by massive hepatocyte necrosis and hepatic dysfunction, and it has a high fatality rate [9,10]. Patients with NAFLD are at a several times higher risk of developing hepatocellular carcinoma (HCC) than the general population [11,12]. As one of the most common chronic liver diseases worldwide, NAFLD imposes a heavy physical burden on patients, as its shackles on people’s bodies and lives is...
similar to a chain [13,14]. Moreover, NAFLD imposes a severe financial burden on patients and their families [15]. This paper systematically introduces the generation and metabolic pathways of H2S in vivo, various types of donors currently used for H2S release, and study evidence of H2S improvement effects on NAFLD.

2. Generation and Metabolic Pathways of H2S
2.1. Metabolism and Production of H2S In Vivo
2.1.1. Metabolism of H2S In Vivo

Unlike NO, H2S is relatively stable in body fluids. In the circulation or cytoplasm, free H2S can be scavenged by oxidation, methylation, and binding to methaemoglobin and excreted as gas or urine (Figure 1). To maintain the physiological balance of H2S to limit the adverse effects of H2S, four H2S decomposition pathways have been detected in mammals [16,17]. First, free H2S can be methylated to yield methanethiol (CH3-SH) and dimethylsulfide (DMS, CH3-S-CH3) by thiol S-methyltransferase in the cytoplasm [18]. Second, H2S is initially oxidized by sulphide quinone oxidoreductase (SQR), generating an SQR-bound persulfide intermediate. Then, the SQR-bound persulfide is transferred to an acceptor, such as GSH, yielding a molecule of oxidized glutathione (GSSG) in this process. Moreover, GSSH is converted to sulphite (SO32−) by ethylmalonic encephalopathy protein 1 (ETHE1). The formed sulphite can either be converted into thiosulphate (S2O32−) by thiosulphate sulfurtransferase, or it can be directly used by sulphite oxidase (SUOX) to generate sulphate (SO42−) [19,20]. Third, H2S can be scavenged by methemoglobin, forming green sulfhemoglobin [20–22]. However, the mechanism of H2S binding to hemoglobin is not clear. Finally, these sulfur-containing substances can be excreted from the body as exhaled H2S gas or urine containing thiosulfate, sulfate, and dimethylsulfide (DMS, CH3-S-CH3) by thiol S-methyltransferase in the cytoplasm [18].

2.1.2. H2S Production In Vivo

Liver H2S is considered to be derived from endogenous liver synthesis and exogenous sources from the gastrointestinal tract. Exogenously, the gut microbiota is the major producer of H2S in vivo. In fact, the H2S level in sterile mice is 80% lower than in conventional mice [24]. L-cysteine can be metabolized in vivo to produce H2S through the cysteine desulphhydrase from cysteine-desulfurizing bacteria, such as E. coli and S. enterica [25]. In addition, sulphate-reducing bacteria (SRB), including Desulfovibrio, Desulfovacter, and Desulfotomaculum, can also produce H2S via the reduction of inorganic sulphate or microbial catabolism of sulphomucins. As H2S is mainly produced from sulfur-containing
amino acids, a high protein diet (HPD) can significantly change microbiota composition to increase the amount of H$_2$S-producing bacteria [26]. In fact, several studies have revealed that mice fed with HPD exhibited an increase in sulphate-reducing bacterial abundance and higher amounts of colonic H$_2$S [27]. Meanwhile, individuals fed with HPD for ten days had a 15-fold improvement in fecal sulfide compared with those fed with a vegetarian diet [28]. In other studies, Attene-Ramos et al. have shown in vitro that H$_2$S in excess is detrimental for colonic epithelium energy metabolism and DNA integrity. However, intestinal cells can improve the H$_2$S oxidation capacity in mitochondria to limit the adverse effects of H$_2$S [29]. Furthermore, non-enzymatic H$_2$S production pathways have been widely reported in mammals. Koj et al. reported that H$_2$S was produced when rat liver mitochondria were incubated with oxygen, glutathione (GSH), and thiosulphate [30]. Studies have also demonstrated that coordinated catalysis of cysteine with ferric iron and vitamin B6 result in dose-dependent intra-vascular release of abundant H$_2$S [31]. It has also been considered that a non-enzymatic H$_2$S generation pathway may occur in the liver, which will hold high levels of iron storage.

According to a number of recent studies, there are four enzymatic pathways to generate H$_2$S in mammals, namely the cystathionine $\beta$-synthase (CBS) pathway, the cystathionine $\gamma$-lyase (CSE) pathway, the 3-mercaptoppyruvate sulfurtransferase (3-MST)/cysteine aminotransferase (CAT) pathway, and the 3-MST/D-amino acid oxidase (DAO) pathway (Figure 2) [32–34]. CBS and CSE are the main enzymes that produce H$_2$S in the liver, using L-cysteine, L-homocysteine, or L-cystathionine as the major substrates [35,36]. CBS is primarily expressed in the central nervous system and liver, while CSE is mainly located in the vascular system, liver, and kidney [37–40]. CBS, a unique heme-containing enzyme, can catalyze the pyridoxal-5-phosphate-dependent condensation of DL-homocysteine (DL-HCY) with serine to form L-cystathionine and water [41,42]. Then, L-cystathionine can be catalyzed by CSE to dimerize into L-cystine. L-cystine can be catalyzed by CSE or CBS via a $\beta$ elimination reaction to yield H$_2$S [43,44]. Moreover, 3-MST, along with CAT, is an important pathway for the production of H$_2$S in mitochondria [45]. In the presence of CAT, L-cysteine can be catalyzed to transfer its amine group to $\alpha$-ketoglutarate forming 3-mercaptoppyruvate (3-MP) and glutamate. Then, 3-MP can be catalyzed by 3-MST to produce H$_2$S [46,47]. It is worth mentioning that pyridoxal-5-phosphate is an indispensable cofactor for synthesizing H$_2$S by CSE and CBS, while 3-MST requires zinc as a cofactor to synthesize H$_2$S. Additionally, though CSE and CBS are mainly localized in the cytoplasm, they can translocate into mitochondria under certain oxidative conditions, whereas 3-MST usually reside and produce H$_2$S in mitochondria. The DAO/3-MST pathway is the fourth H$_2$S generation pathway in vivo, which was first discovered by Kimura et al. [32]. They found that kidney lysates can produce more than 60-fold H$_2$S by using D-cysteine as a substrate compared with L-cysteine [48]. D-cysteine is oxidized to 3-MP, ammonia (NH$_3$), and hydrogen peroxide (H$_2$O$_2$) in the presence of DAO. Then, 3-MP is introduced into mitochondria and metabolized by 3-MST to produce H$_2$S. As DAO is only located in the brain and kidney, this H$_2$S generation pathway is believed to exclusively exist in the above mentioned two organs [49].

### 2.2. Development of H$_2$S-Based Therapeutics

Along with the demonstrated therapeutic effect, the development of H$_2$S-based therapeutics relies on physiologically stable H$_2$S donors, which can deliver H$_2$S to the desired locations at the appropriate concentrations. Direct inhalation obviously would not be an acceptable approach for many reasons including smell, irritation, and enhanced local concentrations at the lung. More recently, an increasing number of H$_2$S donors, as well as their H$_2$S-related biological effects, have been reported [50]. In addition to natural H$_2$S donors [51], more organic synthetic H$_2$S donors have also been developed [52] (Table 1). In general, H$_2$S donors can be classified as natural sulfur-containing organic compounds, inorganic sulfide salts, small-molecule synthetic organic compounds, and DTT (1, 2-dithiole-3-thiones)–coupled non-steroidal anti-inflammatory drugs (NSAIDs).
2.2.1. Natural Sulfur-Containing Organic Compounds

Garlic and onions are recognized as the main source of natural H\textsubscript{2}S donors. Sulfur-containing organic compounds derived from garlic are generally byproducts of the breakdown of thiosulfinates (R-SO\textsubscript{2}-SR). Among them, Allicin, as the most common form of the thiosulfinates, can be decomposed into four different types of H\textsubscript{2}S-releasing compounds, namely diallyl sulfide (DAS), diallyl disulfide (DADS), S-allylcysteine (SAC), and diallyl trisulfide (DATS) [53,54]. The above-mentioned garlic-derived H\textsubscript{2}S donors can be converted into H\textsubscript{2}S by human red blood cells in the presence of naturally free thiols, such as homocysteine, GSH, N-acetylcysteine, and cysteine [55]. In cruciferous plants, the natural glucosinolates can be catalyzed by myrosinase to generate isothiocyanates (ITCs), which are H\textsubscript{2}S-releasing compounds, and they have preventive and therapeutic effects on various types of diseases. The limitations of these natural H\textsubscript{2}S donors are that they have poor water solubility and generate various byproducts after the H\textsubscript{2}S release [56].

2.2.2. Inorganic Sulfide Salts

Inorganic sulfide salts, including CaS, Na\textsubscript{2}S, and NaHS, etc., have most widely been studied as H\textsubscript{2}S donors in medical studies [57–60]. Sulfide salts are generally solid analogs of the gas, which exist in the form of HS\textsuperscript{-} anions and H\textsubscript{2}S molecules under the physiological condition. Na\textsubscript{2}S and NaHS (particularly NaHS) have been most commonly used to assess the therapeutic potential of exogenous H\textsubscript{2}S delivery, and CaS has been proven to be a more stable H\textsubscript{2}S donor reagent than Na\textsubscript{2}S and NaHS [61]. Physiological studies on H\textsubscript{2}S using sulfide salts usually need high-dose treatment, leading to a surge in H\textsubscript{2}S concentrations in the blood and tissues to physiological levels and then a rapid decline in H\textsubscript{2}S levels. This delivery strategy is significantly different from that of endogenous H\textsubscript{2}S generation in which concentrations are tightly regulated. These drawbacks make it necessary to explore novel H\textsubscript{2}S-donors continuously to control the dose, duration, timing, and location of H\textsubscript{2}S release.

2.2.3. Lawesson’s Reagent

Lawesson’s reagent, a well-known H\textsubscript{2}S donor, can generate H\textsubscript{2}S in aqueous media over a considerably longer period than sulfide salts. However, Lawesson’s reagent has not been widely used as an H\textsubscript{2}S donor by researchers because of its poor water solubility. GYY4137, a high water-soluble derivative of Lawesson’s reagent, can generate H\textsubscript{2}S via hydrolysis [62]. Owing to its commercial availability and high water solubility, GYY4137 is the most

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*Figure 2. Pathway of H\textsubscript{2}S production in mammalian cells.*
extensively used H₂S donor aside from sulfide itself. GYY4137 has been demonstrated to be a valuable H₂S-releasing compound for researchers, particularly in a study of the effect of the H₂S release rate on physiological outcomes. However, GYY4137 has an obvious disadvantage. It is usually prepared and sold as a dichloromethane complex, which is residual after crystallization. Dichloromethane can be metabolized again to produce CO, which is another gas signal molecule with a similar biological effect to that of H₂S. Thus, the effects produced by GYY4137 may be attributed to CO [63,64].

2.2.4. Dithiolthiones

1,2-Dithiole-3-thiones (DTTs) are a class of small-molecule synthetic organic H₂S donors [65,66]. DTTs can be easily synthesized through the reaction of anethole with elemental sulfur. DTTs are commonly viewed as one of the hydrolysis-triggered H₂S donors, which are capable of being easily linked to other molecules to prepare drug-DTT conjugates. The DTT moiety has usually been appended to non-steroidal anti-inflammatory drugs (NSAIDs) and studied rather extensively, such as HS-Aspirins, HS-Sulindac, HS-Naproxen, HS-Diclofenac, HS-Mesalamine (ATB-429), and HS-Indomethacin (ATB-43) [67]. However, two problems must be taken seriously when such compounds are used to investigate the biological function of H₂S. First, it is not clear whether DTT can release sufficient H₂S under physiological conditions. Second, anethole trithione (ADT), one of the DTT derivatives, is widely used for coupling other drugs to produce H₂S-donating versions of these drugs. However, it is still unclear whether the biological function of ADT itself or the H₂S released by ADT plays a role.

Table 1. Summary of current H₂S donors.

| H₂S Donors                        | Chemical Compound                  | Bioactivity                                      | Drawbacks                                      | Ref.   |
|-----------------------------------|------------------------------------|--------------------------------------------------|------------------------------------------------|--------|
| Inorganic salts                   | NaHS/CaS/NaS₂                       | Anti-inflammation, cardioprotective effects, diabetes amelioration | Action time short, uncontrollable              | [68]   |
| Lawesson’s reagent                | GYY4137                            | Anti-inflammation, vasodilation                   | Slow hydrolysis rate, metabolized to CO        | [62,69]|
| DTTs                              | ADT-OH                              | Reducing cell viability                           | Increasing arterial pressure                   | [70]   |
|                                  | DTT-NSAID                           | Anti-inflammation                                 | Poor selectivity                               | [71]   |
| Derivatives of Allium sativum extracts | DATS/DADS                         | Regulating blood vessels                          | Poor water solubility, generating byproducts   | [72]   |
|                                  | SPRC                                | Anti-inflammation, anti-oxidation                 | Unstable and short half-life                   | [55,73]|
| Derivatives of thioamino acids    | Thioglycine/Thiovaline              | Vasodilation                                      | Poor selectivity, slow release rate            | [74]   |
| Derivatives of anti-inflammatory drugs | S-aspirin                          | Anti-inflammation, cardiovascular protection      | Complications in the upper gastrointestinal tract | [71]   |
| Derivatives of anti-inflammatory drugs | S-diclofenac                     | Anti-inflammation, gastrointestinal protection    | High cardiovascular risk                       | [75]   |
| Derivatives of anti-inflammatory drugs | ATB-429                            | Anti-inflammation                                 | Increasing arterial pressure                   | [76]   |
| Derivatives of anti-inflammatory drugs | ATB-346                            | Anti-inflammation, antipyretic, analgesic         | Increasing arterial pressure                   | [77]   |
| Thiol-triggered donors            | N-Benzoylthiobenzamides             | Cardioprotection                                  | Poor selectivity                               | [78]   |
| Thiol-triggered donors            | Acyl perthiols                      | Cardioprotection                                  | Poor selectivity                               | [79]   |
Table 1. Cont.

| H₂S Donors                  | Chemical Compound                  | Bioactivity          | Drawbacks             | Ref.  |
|-----------------------------|------------------------------------|----------------------|-----------------------|-------|
| Thiol-triggered donors      | Dithioperoxyanhydrides             | Vasodilation         | Poor selectivity      | [80]  |
| Thiol-triggered donors      | Arylthioamides                     | Vasodilation         | Poor selectivity      | [81]  |
| Thiol-triggered donors      | S-Aroylthiooximes                  | Anti-cancer proliferation | Poor selectivity   | [82]  |
| Photosensitive H₂S Donor    | Geminal-dithiols                   | Restores anti-microbial resistance | Poor selectivity | [83]  |
| Photosensitive H₂S Donor    | Ketoprofenate photocages            | Unknown              | Poor selectivity      | [84]  |
| Photosensitive H₂S Donor    | α-Thioetherketones                 | Anti-inflammation    | Poor selectivity      | [85]  |
| Enzyme-triggered H₂S donor  | BW-HP-101                          | Esterase triggered, anti-inflammation | Unknown            | [86]  |
| pH-triggered H₂S donor      | JK-1/JK-2                          | MI/R protection      | Unknown               | [87]  |
| Dual COS/H₂S donor          | N-Thiocarboxyanhydrides             | Angiogenesis         | Unknown               | [88]  |
| Dual COS/H₂S donor          | Arylboronate thiocarbamates        | Cardioprotection     | Unknown               | [89]  |
| Dual COS/H₂S donor          | α-Nitrobenzyl thiocarbamates       | Unknown              | Unknown               | [90]  |

3. Association between H₂S Level and NAFLD In Vivo

The dominant H₂S generation enzymes in liver tissues depend on CSE rather than CBS and 3-MST enzymes. In NAFLD patients, the expression of hepatic CSE was significantly down-regulated by approximately 33% compared to that in non-NAFLD patients [91]. In line with the CSE down-regulation, the circulating cysteine and homocysteine were increased in NAFLD patients [92]. In mouse hepatocytes treated with OA, the production of H₂S was reduced remarkably, accompanied by the formation of a large number of intracellular lipid droplets detected by lipid staining. Meanwhile, CSE expression was decreased significantly, but not the CBS and 3-MST enzymes [93]. In primary hepatocytes, CSE deficiency increased the formation of lipid droplets, which could be reversed by NaHS treatment.

In an HFD-induced NAFLD mouse, the CSE mRNA and protein expression was decreased by IHC staining [94,95]. In keeping with the CSE down-regulation, the hepatic H₂S generation of the NAFLD mouse was also reduced. The H₂S donor treatment, NaHS and GYY4137, dramatically attenuated HFD-induced steatosis by HE staining, as well as lowered the liver triglyceride level and cholesterol level [96]. Similarly, methionine-choline-deficient (MCD)-induced damage to NASH rats can be prevented with the treatment of H₂S and its release agents [97]. Coinciding with the aforementioned changes, the down-regulation of fatty acid de novo synthesis associated genes (SREBP-IC, ACC, FAS, and SCD-1) was detected [98].

In the hepatocyte-specific CSE deletion mouse model was observed an approximately 75% decrease in the H₂S generation, along with a relatively severe hepatic steatosis development [99]. Accordingly, the triglyceride and total cholesterol level was increased in CSE-knockout mice. In contrast, an enhancement of the CSE activity can inhibit lipid accumulation in hepatocytes [100]. A combination of glucose tolerance tests, insulin tolerance tests, and pyruvate tolerance tests in CSE-knockout mice suggested that the CSE deficiency exacerbated glucose homeostasis and insulin resistance.

4. Physiological Mechanism of H₂S in Alleviating NAFLD

The pathological processes of NAFLD are closely related to lipid metabolism, autophagy, endoplasmic reticulum stress, oxidative stress, inflammation, etc. Meanwhile, a few reports have indicated that H₂S can alleviate NAFLD by regulating these pathological processes.
Autophagy is a highly complex process of cellular degradation or organelle degradation. Dysfunctional autophagy is correlated with several diseases, including cancer, immune dysfunction, and NAFLD. It has been reported that H$_2$S and its donors regulate autophagy through a few molecular mechanisms of alleviating NAFLD, such as the AMPK-mTOR pathway, PI3K/Akt/mTOR signaling pathway, and the Mir-30c signaling pathway (Figure 3).

![Figure 3. Mechanism of H$_2$S regulation of autophagy in mammals.](image)

### 4.1.1. AMPK-mTOR Pathway

A potential mechanism of H$_2$S ameliorating NAFLD was the simulation of liver autophagy by H$_2$S through the AMPK-mTOR signal pathway [17]. AMPK is an essential initiator of autophagy, sensing ATP starvation, and cellular energy homeostasis. Its downstream regulatory proteins include the negative-regulation of the mTOR, whose down-regulation enhances autophagosome generation [101]. In contrast, suppression of the AMPK activation inhibits autophagy ability and results in the development of NAFL [102–104]. Similarly, knocking out AMPK in liver cell lines by siRNA blocks the pro-autophagy effect of NaHS. Mice treated with HFD generated a higher level of p-mTOR than those fed with a normal chow diet (NCD). In contrast, treatment with H$_2$S reduced the phosphorylation and thus inhibited mTOR activation [105]. NaHS could decrease serum TG levels of HFD mice, which could be reversed via treatment with chloroquine (CQ), a well-known inhibitor of autophagy [106]. Moreover, NaSH enhanced the phosphorylation of AMPK and thus diminished the p-mTOR in a Western blot analysis. HFD treatment inhibited the phosphorylation of AMPK, which could be abolished through the co-administration of NaSH. Knock-down of the AMPKα2 subunit in mice inhibited the autophagic improvement effects of NaSH.

### 4.1.2. PI3K/Akt/mTOR Signaling Pathway

The PI3K/Akt/mTOR signaling pathway is a vital pathway correlated with the regulation of autophagy by H$_2$S [107]. It has been proven that H$_2$S enhances autophagy by suppressing reactive oxygen species (ROS)-mediated PI3K/AKT/mTOR cascade in OA-induced LO2 cells [108]. In addition, NaHS and rapamycin significantly inhibit the protein expression of PI3K, Akt, and mTOR in HCC cells, indicating that the autophagy improvement by H$_2$S is mainly initiated via the PI3K/AKT/mTOR signaling pathway. A number of studies have reported that high concentrations of H$_2$S restraints the gene
expression correlated with the PI3/Akt/mTOR pathway and increases the expression of other autophagy-related proteins, such as Beclin1, ATG5, and the ratio of LC3-II/LC3-I. It has been exhibited that inhibiting the PI3K/AKT signaling pathway via LY294002 reduces the improvement effect of H2S against scratch-induced cellular ROS level and NRF2 accumulation in the nucleus. The increased levels of autophagy in hepatocytes significantly enhance lipolysis and reduce the stress caused by fat accumulation in the liver [17,109].

4.1.3. Mir-30c Signaling Pathway

Autophagy can also be induced by H2S via the mir-30c pathway [110]. Treatment with H2S can down-regulate mir-30c expression and up-regulate Beclin-1 and LC3 expressions. The in vitro experiments exhibited that mir-30c negatively regulated the Beclin-1 expression in cells by targeting the 3'UTR region. However, pre-incubation with the autophagy inhibitor 3-Methyladenine (3-MA) can eliminate the protective effect of H2S. These results suggest that H2S can play an autophagy role by inhibiting mir-30c and up-regulating Beclin-1 [110].

4.2. H2S Alleviates NAFLD by Regulating Inflammation

Numerous studies have demonstrated that H2S exerts various anti-inflammatory effects in tissues, including the liver tissues of patients with NAFLD. A previous study demonstrated that NaHS protected hepatocytes from PA-induced inflammatory damage through the down-regulation of the secretion of TNF-α, IL-6, IL-1β, and NLRP3. The LPS-induced RAW264.7 cells treated with NaHS can significantly decrease the protein expression of CX3CR1, an essential chemokine receptor in an inflammatory response (Figure 4). Meanwhile, accumulating evidences suggest that an over-expression of CSE contrary evidence shows that H2S exposure increases the expression of necrosis-related genes (RIPK1, RIPK3, MLKL, TAK1, and TAB3) and induces the TNF-α release, exhibiting an inflammatory response [113]. This inconsistency in the H2S treatment is attributed to the difference in H2S concentration.

4.3. H2S Alleviates NAFLD by Improving Oxidative Stress

Although the mechanism of oxidative stress in hepatocytes is particularly complex, it is generally characterized by increased ROS production and insufficient scavenging of ROS through endogenous antioxidant defense. Oxidative stress is an essential factor in the

Figure 4. H2S alleviates NAFLD by improving inflammation, oxidative stress, lipid and glucose metabolism, and ER stress. Upward arrows indicate up-regulation of gene expression.
progression of NAFLD [98]. Therefore, regulating oxidative stress in hepatocytes is a potential strategy for treating NAFLD. Emerging data indicate that the palmitic acid-induced NAFLD cell model exhibits a high level of ROS by cell fluorescence detection. However, the over-expression of CES and CBS can decrease the ROS levels in a concentration-dependent manner. HFD feeding significantly boosts the generation of malondialdehyde (MDA), which is the final product of lipid peroxidation and serves as a biomarker of oxidative stress. A few studies have reported that an NaHS treatment can significantly reduce the formation of liver MDA and increase the activity of antioxidant enzymes [114]. Recent evidence has revealed that treatment with a low concentration of an H\(_2\)S donor (NaHS or Na\(_2\)S) can reduce lipid peroxidation levels in hepatocytes and increase the activity of antioxidant enzymes such as GSTs (Figure 4) [115]. It has also been demonstrated that H\(_2\)S can inhibit the transcriptional activity of NF-kB, resulting in sulfhydration of Kelch-like ECH-associated protein 1 (KEAP1); the activated KEAP1 then releases active nuclear factor erythroid 2-related factor 2 (NRF2), causing an increased expression of antioxidant-response elements [116,117].

4.4. H\(_2\)S Regulates Lipid and Glucose Metabolism

Several studies have proved that H\(_2\)S can be severed as a crucial regulator of the hepatic lipid and glucose metabolism in NAFLD. The lack of endogenous H\(_2\)S is the vital pathogenesis of dyslipidemia and hyperglycemia. Cai et al. found that H\(_2\)S increases the triglyceride accumulation in mice fed with an HFD and weakens the insulin resistance of adipose tissues. Peroxisome proliferator-activated receptors \(\gamma\) (PPAR\(\gamma\)) are ligand-activated nuclear receptors that regulate glucose and lipid metabolism. Endogenous H\(_2\)S can enhance the PPAR\(\gamma\) activity via the sulfhydration at the C139 site, thus increasing the glucose uptake and lipid storage in adipocytes [118]. Farnesoid X receptor (FXR), a type of nuclear receptor, plays an essential role in the pathological process of NAFLD by attenuating steatosis and enhancing insulin sensitivity [119]. Several studies have demonstrated that CSE knockout can decrease the FXR mRNA and protein levels [91]. In contrast, overexpression of CSE or NaHS treatment can increase the FXR mRNA and protein levels. Furthermore, NaHS can promote the FXR sulfhydration at the Cys138/141 sites, thus increasing its activity to regulate the expression of the target genes correlated with glucose and lipid metabolism. These essential regulatory gene coding proteins consist of ACC, FAS and stearoyl-CoA desaturase 1 (SCD1) (Figure 4).

4.5. H\(_2\)S Alleviates NAFLD by Improving ER Stress

The endoplasmic reticulum (ER) is a crucial organelle that provides a field for the folding and modifying of proteins. Excessive unfolded proteins in the ER will result in a situation called ER stress, which can be initiated through a series of internal or external environmental changes, including aging, environmental factors, and/or genetic mutations. Previous transcriptome data have indicated that the total protein expression in patients with NAFLD was significantly reduced compared to normal individuals [120]. The phosphatase PTP1B, located at the rough ER in the cytoplasm, plays an essential role in ER signaling. Several studies have demonstrated that a high endogenous H\(_2\)S level can reduce ER stress by the sulfhydration of PTP1B [121,122]. The phosphorylation of eIF2\(\alpha\), leading to decreased protein synthesis, is a crucial biochemical step for ER stress. Yadav et al. have also reported that H\(_2\)S can decrease the dephosphorylation of the eukaryotic translation initiation factor 2\(\alpha\) (eIF2\(\alpha\)) via the sulfhydration of PPIc at Cys127 and thus regulate the ER stress (Figure 4) [123,124].

5. Conclusions

In this work, we systematically described the pathway of H\(_2\)S generation and metabolic pathways in vivo, the different types of H\(_2\)S donors, and their effects on NAFLD. Accumulating evidence indicated that the H\(_2\)S level was strikingly correlated with NAFLD in various physiological processes [125]. Whether the models were fatty acid-induced cell
or high-fat diet induced animal, H\(_2\)S undoubtedly exhibited its vital role in improving NAFLD. However, the stability and safety of conventional H\(_2\)S donors could not meet the requirements of medical usage. Therefore, novel H\(_2\)S donors with strong selectivity and safety remain required. The recent emergences of light-triggered H\(_2\)S donors, pH-triggered H\(_2\)S donors, and dual COS/H\(_2\)S donors have provided a new strategy for the clinical application of H\(_2\)S. Moreover, new mechanisms of H\(_2\)S on improving NAFLD have been constantly updated in recent years. Increasing evidence indicates that modifying specific cysteine in target proteins via sulfhydration, including various enzymes and transcription factors, is an important mechanism for regulating the different pathological processes of NAFLD.

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**References**

1. Malone Rubright, S.L.; Pearce, L.L.; Peterson, J. Environmental toxicology of hydrogen sulfide. *Nitric Oxide* 2017, 71, 1–13. [CrossRef] [PubMed]
2. Daldal, H.; Beder, B.; Serin, S.; Sungurtekin, H. Hydrogen sulfide toxicity in a thermal spring: A fatal outcome. *Clin. Toxicol.* 2010, 48, 755–756. [CrossRef]
3. Perna, A.F.; Luciano, M.G.; Ingrosso, D.; Raiola, I.; Pulzella, P.; Sepe, I.; Lanza, D.; Violettei, E.; Capasso, R.; Lombardi, C.; et al. Hydrogen sulfide, the third gaseous signaling molecule with cardiovascular properties, is decreased in hemodialysis patients. *J. Ren. Nutr.* 2010, 20, S11–S14. [CrossRef]
4. Kolesnikov, S.I.; Vlasov, B.Y.; Kolesnikova, L.I. Hydrogen Sulfide as a Third Essential Gas Molecule in Living Tissues. *Vestn. Ross. Akad. Meditsinskikh Nauk.* 2015, 70, 237–241. [CrossRef]
5. Zhou, Y.; Ding, Y.L.; Zhang, J.L.; Zhang, P.; Wang, J.Q.; Li, Z.H. Alpinetin improved high fat diet-induced non-alcoholic fatty liver disease (NAFLD) through improving oxidative stress, inflammatory response and lipid metabolism. *Biomed. Pharm.* 2018, 97, 1397–1408. [CrossRef]
6. Hirsova, P.; Bohm, F.; Dohnalkova, E.; Nozickova, B.; Heikenwalder, M.; Gores, G.J.; Weber, A. Hepatocyte apoptosis is tumor promoting in murine nonalcoholic steatohepatitis. *Cell Death Dis.* 2020, 11, 80. [CrossRef]
7. Guo, L.; Guo, Y.Y.; Li, B.Y.; Peng, W.Q.; Chang, X.X.; Gao, X.; Tang, Q.Q. Enhanced acetylation of ATP-citrate lyase promotes the progression of nonalcoholic fatty liver disease. *J. Biol. Chem.* 2019, 294, 11805–11816. [CrossRef]
8. Kashyap, M.L.; Ganji, S.; Nakra, N.K.; Kamanna, V.S. Niacin for treatment of nonalcoholic fatty liver disease (NAFLD): Novel use for an old drug? *J. Clin. Lipidol.* 2019, 13, 873–879. [CrossRef]
9. Seko, Y.; Yamaguchi, K.; Itoh, Y. The genetic backgrounds in nonalcoholic fatty liver disease. *Clin. J. Gastroenterol.* 2018, 11, 97–102. [CrossRef]
10. Han, C.Y.; Rho, H.S.; Kim, A.; Kim, T.H.; Jang, K.; Jun, D.W.; Kim, J.W.; Kim, B.; Kim, S.G. FXR Inhibits Endoplasmic Reticulum Stress-Induced NLRP3 Inflammasome in Hepatocytes and Ameliorates Liver Injury. *Cell Rep.* 2018, 24, 2985–2999. [CrossRef]
11. Margini, C.; Dufour, J.F. The story of HCC in NAFLD: From epidemiology, across pathogenesis, to prevention and treatment. *Liver. Int. Off. J. Int. Assoc. Study Liver* 2016, 36, 317–324. [CrossRef] [PubMed]
12. Wu, W.K.K.; Zhang, L.; Chan, M.T.V. Autophagy. NAFLD and NAFLD-Related HCC. *Adv. Exp. Med. Biol.* 2018, 1061, 127–138. [PubMed]
13. Estes, C.; Anstee, Q.M.; Arias-Loste, M.T.; Bantel, H.; Bellentani, S.; Caballeria, J.; Colombo, M.; Craxi, A.; Crespo, J.; Day, C.P.; et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. *J. Hepatol.* 2018, 69, 896–904. [CrossRef]
14. Kumar, R.; Priyadarshi, R.N.; Anand, U. Non-alcoholic Fatty Liver Disease: Growing Burden, Adverse Outcomes and Associations. *J. Clin. Transl. Hepatol.* 2020, 8, 76–86. [CrossRef]

15. Shao, Y.L.; Fan, J.G. Prevalence and harm of nonalcoholic fatty liver disease. *Zhonghua Gan Zang Bing Za Zhi* 2019, 27, 10.

16. Xu, S.; Liu, Z.; Liu, P. Targeting hydrogen sulfide as a promising therapeutic strategy for atherosclerosis. *Int. J. Cardiol.* 2014, 172, 313–317. [CrossRef]

17. Wu, D.; Wang, H.; Teng, T.; Duan, S.; Ji, A.; Li, Y. Hydrogen sulfide and autophagy: A double edged sword. *Pharmacol. Res.* 2018, 131, 120–127. [CrossRef]

18. Wang, R. Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. *Physiol. Rev.* 2012, 92, 791–896. [CrossRef]

19. Bostelaar, T.; Vitvitsky, V.; Kumutima, J.; Lewis, B.E.; Yadav, P.K.; Brunold, T.C.; Filipovic, M.; Lehnert, N.; Stemmler, T.L.; Banerjee, R. Hydrogen Sulfide Oxidation by Myoglobin. *J. Am. Chem. Soc.* 2016, 138, 8476–8488. [CrossRef]

20. Jensen, B.; Fago, A. Reactions of ferric hemoglobin and myoglobin with hydrogen sulfide under physiological conditions. *J. Inorg. Biochem.* 2018, 182, 133–140. [CrossRef]

21. Zuhra, K.; Tomé, C.S.; Masi, L.; Giardina, G.; Paulini, G.; Maligranò, F.; Forte, E.; Vicente, J.B.; Giumfrè, A. N-Acetylcysteine Serves as Substrate of 3-Mercaptopyruvate Sulfurtransferase and Stimulates Sulfide Metabolism in Colon Cancer Cells. *Cells* 2019, 8, 828. [CrossRef]

22. Kashfi, K. The dichotomous role of H2S in cancer cell biology? Deja vu all over again. *Biochem. Pharm.* 2018, 149, 205–223. [CrossRef] [PubMed]

23. Hildebrandt, T.M.; Grieshaber, M.K. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J.* 2008, 275, 3352–3361. [CrossRef]

24. Shen, X.; Carlström, M.; Borniquel, S.; Fäldt, C.; Kevil, C.G.; Lundberg, J.O. Microbial regulation of host hydrogen sulfide bioavailability and metabolism. *Free. Radic. Biol. Med.* 2013, 60, 195–200. [CrossRef]

25. Awano, N.; Wada, M.; Mori, H.; Nakamori, S.; Takagi, H. Identification and functional analysis of Escherichia coli cysteine desulphydrases. *Appl. Environ. Microbiol.* 2005, 71, 4149–4152. [CrossRef]

26. Khalil, N.A.; Walton, G.E.; Gibson, G.R.; Tuohy, K.M.; Andrews, S.C. In vitro batch cultures of gut microbiota from healthy and ulcerative colitis (UC) subjects suggest that sulphate-reducing bacteria levels are raised in UC and by a protein-rich diet. *Int. J. Food Sci. Nutr.* 2014, 65, 79–88. [CrossRef] [PubMed]

27. Mu, C.; Yang, Y.; Luo, Z.; Guan, L.; Zhu, W. The Colonic Microbiome and Epithelial Transcriptome Are Altered in Rats Fed a High-Protein Diet Compared with a Normal-Protein Diet. *J. Nutr.* 2016, 146, 474–483. [CrossRef] [PubMed]

28. Magee, E.A.; Richardson, C.J.; Hughes, R.; Cummings, J.H. Contribution of dietary protein to sulfide production in the large intestine: An in vitro and a controlled feeding study in humans. *Am. J. Clin. Nutr.* 2000, 72, 1488–1494. [CrossRef]

29. Beaumont, M.; Andriamihaja, M.; Lan, A.; Khodorova, N.; Audebert, M.; Blouin, J.M.; Grauso, M.; Lancha, L.; Benetti, P.H.; Benamouzig, R.; et al. Detrimental effects for colonocytes of an increased exposure to luminal hydrogen sulfide: The adaptive response. *Free. Radic. Biol. Med.* 2016, 93, 155–164. [CrossRef]

30. Koj, A.; Frendo, J.; Janik, Z. [35S]thiosulphate oxidation by rat liver mitochondria in the presence of glutathione. *Biochem. J.* 1967, 103, 791–795. [CrossRef] [PubMed]

31. Yang, J.; Minkler, P.; Grove, D.; Wang, R.; Willard, B.; Dweik, R.; Hine, C. Non-enzymatic hydrogen sulfide production from cysteine in blood is catalyzed by iron and vitamin B(6). *Commun. Biol.* 2019, 2, 194. [CrossRef]

32. Shibuya, N.; Koike, S.; Tanaka, M.; Ishigami-Yuasa, M.; Kimura, Y.; Ogasawara, Y.; Fukui, K.; Nagahara, N.; Kimura, H. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat. Commun.* 2013, 4, 1366. [CrossRef]

33. Stipanuk, M.H.; Beck, P.W. Characterization of the enzymic capacity for cysteine desulphydrization in liver and kidney of the rat. *Biochem. J.* 1982, 206, 267–277. [CrossRef]

34. Shibuya, N. Production of H(2)S, H(2)S(n), and persulfide species (CysSSH and GSSH) by 3-mercaptopyruvate sulfurtransferase. *Nihon Yakurigaku Zasshi Folia Pharmacol. Jpn.* 2018, 152, 216–222. [CrossRef]

35. Renga, B. Hydrogen sulfide generation in mammals: The molecular biology of cystathionine-β- synthase (CBS) and cystathionine-γ-lyase (CSE). *Inflamm. Allergy Drug Targets* 2011, 10, 85–91. [CrossRef]

36. Yamamoto, J.; Sato, W.; Kosugi, T.; Yamamoto, T.; Kimura, T.; Taniguchi, S.; Kojima, H.; Maruyama, S.; Imai, E.; Matsuo, S.; et al. Distribution of hydrogen sulfide (H2S)-producing enzymes and the roles of the H2S donor sodium hydrosulfide in diabetic nephropathy. *Clin. Exp. Nephrol.* 2013, 17, 32–40. [CrossRef]

37. Fagerberg, L.; Hallström, B.M.; Oksvold, P.; Kampf, C.; Djureinovic, D.; Odeberg, J.; Habuka, M.; Tahmasepoo, S.; Danielsson, A.; Edlund, K.; et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol. Cell. Proteom.* 2014, 13, 397–406. [CrossRef]

38. Kimura, H. Signaling by hydrogen sulfide (H(2)S) and polysulfides (H(2)S(n)) in the central nervous system. *Neurochem. Int.* 2019, 126, 118–125. [CrossRef] [PubMed]

39. Emmez, H.; Borcek, A.O.; Gonul, I.I.; Belen, H.B.; Solanoglu, I.; Baykaner, M.K. The Effect of Hydrogen Sulphide on Experimental Cerebral Vasospasm. *Turk. Neurol. Surg.* 2017, 27, 374–379. [CrossRef] [PubMed]

40. Xu, X.; Yan, Q.; Liu, X.; Li, P.; Li, X.; Chen, Y.; Simoncini, T.; Liu, J.; Zhu, D.; Fu, X. 17β-Estradiol nongenomically induces vascular endothelial H(2)S release by promoting phosphorylation of cystathionine-γ-lyase. *J. Biol. Chem.* 2019, 294, 15577–15592. [CrossRef]
41. Chen, X.; Jhee, K.H.; Kruger, W.D. Production of the neuromodulator H2S by cystathionine-beta-synthase via the condensation of cysteine and homocysteine. *J. Biol. Chem.* **2004**, *279*, 52082–52086. [CrossRef]
42. Majtan, T.; Krišt, J.; Sokolova, J.; Křizíková, M.; Rašal, M.A.; Kent, J.; Gregory, J.F., 3rd; Kozich, V.; Kraus, J.P. Biogenesis of Hydrogen Sulfide and Thioethers by Cystathionine Beta-Synthase. *Antioxid. Redox Signal* **2018**, *28*, 311–323. [CrossRef]
43. Braunstein, A.E.; Goryachenkova, E.V.; Tolosa, E.A.; Willhardt, I.H.; Yefremova, I.L. Specificity and some other properties of liver serine sulphhydrylase: Evidence for its identity with cystathionine - synthase. *Biochim. Biophys. Acta* **1971**, *242*, 247–260. [CrossRef]
44. Miles, E.W.; Kraus, J.P. Cystathionine beta-synthase: Structure, function, regulation, and location of homocystinuria-causing mutations. *J. Biol. Chem.* **2004**, *279*, 29671–29874. [CrossRef] [PubMed]
45. Yu, X.H.; Cui, L.B.; Wu, K.; Zheng, X.L.; Cayabyab, F.S.; Chen, Z.W.; Tang, C.K. Hydrogen sulfide as a potent cardiovascular protective agent. *Clin. Chim. Acta* **2014**, *437*, 78–87. [CrossRef] [PubMed]
46. Nagahara, N.; Nirasawa, T.; Yoshii, T.; Niimura, Y. Is novel signal transducer sulfur oxide involved in the redox cycle of persulfide at the catalytic site cysteine in a stable reaction intermediate of mercaptopyruvate sulfurtransferase? *Antioxid. Redox Signal.* **2012**, *16*, 747–753. [CrossRef]
47. Nagahara, N.; Koike, S.; Nirasawa, T.; Kimura, H.; Ogasawara, Y. Alternative pathway of H(2)S and polysulfides production from sulffurated catalytic-cysteine of reaction intermediates of 3-mercaptoppyruvate sulfurtransferase. *Biochem. Biophys. Res. Commun.* **2018**, *496*, 648–653. [CrossRef] [PubMed]
48. Kiss, D.J.; Ferenczy, G.G. A detailed mechanism of the oxidative half-reaction of d-amino acid oxidase: Another route for flavin oxidation. *Org. Biomol. Chem.* **2019**, *17*, 7973–7984. [CrossRef]
49. Cao, X.; Bian, J.S. The Role of Hydrogen Sulfide in Renal System. *Front. Pharmacol.* **2016**, *7*, 385. [CrossRef]
50. Nin, D.S.; Binte Idres, S.; Song, Z.; Moore, P.K.; Deng, L.-W. Biological effects of GYY4137 and other phosphorothioate-based hydrogen sulfide donors. *Antioxid. Redox Signal.* **2020**, *32*, 145–158. [CrossRef]
51. Zheng, Y.; Ji, X.; Ji, K.; Wang, B. Hydrogen sulfide prodrugs-a review. *Acta Pharm. Sin. B* **2015**, *5*, 367–377. [CrossRef]
52. Kang, J.; Neill, D.L.; Xian, M. Phosphorothioate-Based Hydrogen Sulfide Releasing Reagents: Chemistry and Biological Applications. *Front. Pharmacol.* **2017**, *8*, 457. [CrossRef] [PubMed]
53. Yao, H.; Luo, S.; Liu, J.; Xie, S.; Liu, Y.; Xu, J.; Zhu, Z.; Xu, S. Controllable thioester-based hydrogen sulfide slow-releasing donors as cardioprotective agents. *Chem. Commun.* **2019**, *55*, 6193–6196. [CrossRef]
54. Cao, X.; Zhang, W.; Moore, P.K.; Bian, J. Protective Smell of Hydrogen Sulfide and Polysulfide in Cisplatin-Induced Nephrotoxicity. *Int. J. Mol. Sci.* **2019**, *20*, 313. [CrossRef]
55. Huang, C.; Kan, J.; Liu, X.; Ma, F.; Tran, B.H.; Zou, Y.; Wang, S.; Zhu, Y.Z. Cardioprotective effects of a novel hydrogen sulfide agent-controlled release formulation of S-propargyl-cysteine on heart failure rats and molecular mechanisms. *PLoS ONE* **2013**, *8*, e69205. [CrossRef]
56. Martelli, A.; Citi, V.; Testai, L.; Brogi, S.; Calderone, V. Organic Isothiocyanates as Hydrogen Sulfide Donors. *Antioxid. Redox Signal.* **2020**, *32*, 110–144. [CrossRef]
57. Zhen, Y.; Wu, Q.; Ding, Y.; Zhang, W.; Zhai, Y.; Lin, X.; Weng, Y.; Guo, R.; Zhang, Y.; Feng, J.; et al. Exogenous hydrogen sulfide promotes hepatocellular carcinoma cell growth by activating the STAT3-COX-2 signaling pathway. *Toxins* **2018**, *10*, 437. [CrossRef] [PubMed]
58. Sousa, F.B.M.; Souza, L.K.M.; Sousa, N.A.; Araujo, T.S.L.; de Araujo, S.; Pacifico, D.M.; Silva, I.S.; Silva, R.O.; Nicolau, L.A.D.; et al. H2S is a key antisepticre molecule against cholera toxin-induced diarrhoea in mice: Evidence for non-involvement of the AC/cAMP/PKA pathway and AMPK. *Nitric Oxide* **2018**, *76*, 152–163. [CrossRef]
59. Malagrinò, F.; Zühra, K.; Mascolo, L.; Mastroncola, D.; Vicente, J.B.; Forte, E.; Giuffré, A. Hydrogen Sulfide Oxidation: Adaptive Changes in Mitochondria of SW 480 Colorectal Cancer Cells upon Exposure to Hypoxia. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 8102936. [CrossRef]
60. Wang, M.; Tang, W.; Zhu, Y.Z. An Update on AMPK in Hydrogen Sulfide Pharmacology. *Front. Pharmacol.* **2017**, *8*, 810. [CrossRef]
61. Hughes, M.N.; Centelles, M.N.; Moore, K.P. Making and working with hydrogen sulfide: The chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: A review. *Free Radic. Biol. Med.* **2009**, *47*, 1346–1353. [CrossRef]
62. Li, L.; Whiteman, M.; Guan, Y.Y.; Neo, K.L.; Cheng, Y.; Lee, S.W.; Zhao, Y.; Baskar, R.; Tan, C.H.; Moore, P.K. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): New insights into the biology of hydrogen sulfide. *Circulation* **2008**, *117*, 2351–2360. [CrossRef]
63. Liu, Z.; Han, Y.; Li, L.; Lu, H.; Meng, G.; Li, X.; Shirhan, M.; Peh, M.T.; Xie, L.; Zhou, S.; et al. The hydrogen sulfide donor, GYY4137, exhibits anti-atherosclerotic activity in high fat fed apolipoprotein E(-/-) mice. *Br. J. Pharmacol.* **2013**, *169*, 1795–1809. [CrossRef]
64. Hou, X.; Yuan, Y.; Sheng, Y.; Yuan, B.; Wang, Y.; Zheng, J.; Liu, C.F.; Zhang, X.; Hu, L.F. GYY4137, an H2S Slow-Releasing Donor, Prevents Nitrative Stress and alpha-Synuclein Nitration in an MPTP Mouse Model of Parkinson’s Disease. *Front. Pharmacol.* **2017**, *8*, 741. [CrossRef]
65. Xie, H.; Xu, Q.; Jia, J.; Ao, G.; Sun, Y.; Hu, L.; Alkayed, N.J.; Wang, C.; Cheng, J. Hydrogen sulfide protects against myocardial ischemia and reperfusion injury by activating AMP-activated protein kinase to restore autophagic flux. *Biochim. Biophys. Res. Commun.* **2015**, *458*, 632–638. [CrossRef]
66. Wallace, J.L.; Caliendo, G.; Santagada, V.; Cirino, G.; Fiorucci, S. Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide-releasing diclofenac derivative in the rat. *Gastroenterology* **2007**, *132*, 261–271. [CrossRef]
67. Kashfi, K. Anti-cancer activity of new designer hydrogen sulfide-donating hybrids. *Antioxid. Redox Signal.* 2014, 20, 831–846. [CrossRef]

68. Zheng, Y.; Yu, B.; De La Cruz, L.K.; Roy Choudhury, M.; Anifowose, A.; Wang, B. Toward Hydrogen Sulfide Based Therapeutics: Critical Drug Delivery and Developability Issues. *Med. Res. Rev.* 2018, 38, 57–100. [CrossRef]

69. Lee, Z.W.; Zhou, J.; Chen, C.S.; Zhao, Y.; Tan, C.H.; Li, L.; Moore, P.K.; Deng, L.W. The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects in vitro and in vivo. *PLoS ONE* 2011, 6, e21077. [CrossRef]

70. Szczesny, B.; Môdis, K.; Yanagi, K.; Coletta, C.; Le Trionnaire, S.; Perry, A.; Wood, M.E.; Whitman, M.; Szabo, C. AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells in vitro. *Nitr. Oxide Biol. Chem.* 2014, 41, 120–130. [CrossRef]

71. Chattopadhyay, M.; Kodela, R.; Nath, N.; Dastagirzadeh, Y.M.; Velázquez-Martínez, C.A.; Boring, D.; Kashfi, K. Hydrogen sulfide-releasing NSAIDs inhibit the growth of human cancer cells: A general property and evidence of a tissue type-independent effect. *Biochem. Pharmacol.* 2012, 83, 715–722. [CrossRef]

72. Benavides, G.A.; Squadrito, G.L.; Mills, R.W.; Patel, H.D.; Isbell, T.S.; Patel, R.P.; Darley-Usmar, V.M.; Doeller, J.E.; Kraus, D.W. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. USA* 2007, 104, 17977–17982. [CrossRef]

73. Pan, L.L.; Liu, X.H.; Gong, Q.H.; Zhu, Y.Z. S-Propargyl-cysteine (SPRC) attenuated lipopolysaccharide-induced inflammatory response in H9c2 cells involved in a hydrogen sulfide-dependent mechanism. *Amino Acids* 2011, 41, 205–215. [CrossRef]

74. Zhou, Z.; van Wantoch Rekowski, M.; Coletta, C.; Szabo, C.; Bucci, M.; Cirino, G.; Topouzis, S.; Papapetropoulos, A.; Giannis, A. Thiolglycine and L-thiovaline: Biologically active H2S donors. *Bioorg. Med. Chem. Med.* 2012, 20, 2675–2678. [CrossRef]

75. Xiao, Z.; Bonnard, T.; Shakouri-Motlagh, A.; Wylie, R.A.L.; Collins, J.; White, J.; Heath, D.E.; Hagemeyer, C.E.; Connal, L.A. Arylthioamides as H2S Donors: L-Cysteine-Activated Releasing Properties and Vascular Effects in Vitro and in Vivo. *Chem. Commun.* 2013, 49, 904–908. [CrossRef]

76. Roger, T.; Raynaud, F.; Bouillaud, F.; Cesbron, M.; Villeneuve, N.; Vilaine, J.P.; Artaud, I.; et al. New biologically active hydrogen sulfide donors. *Chembiochem A Eur. J. Chem. Biol.* 2013, 14, 2268–2271. [CrossRef]

77. Lee, Z.W.; Zhou, J.; Chen, C.S.; Pacheco, A.; Peng, B.; Devarie-Baez, N.O.; Aguilera, H.C.; Lefer, D.J.; et al. Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury. *ACS Chem. Biol.* 2013, 8, 1283–1290. [CrossRef]

78. Zheng, Y.; Yu, B.; Wang, H.; Xian, M. Cysteine-activated hydrogen sulfide (H2S) donors. *Amino Acids* 2013, 45, 1516–1532. [CrossRef]

79. Foster, J.C.; Powell, C.R.; Radzinski, S.C.; Matson, J.B. S-aroylthiooximes: A facile route to hydrogen sulfide releasing compounds with structure-dependent release kinetics. *Org. Lett.* 2014, 16, 1558–1561. [CrossRef]

80. Devarie-Baez, N.O.; Bagdon, P.E.; Peng, B.; Zhao, Y.; Park, C.M.; Xian, M. Light-induced hydrogen sulfide release from “caged” gem-dithiols. *Org. Lett.* 2013, 15, 2786–2789. [CrossRef]

81. Fukushima, N.; Ieda, N.; Sasakura, K.; Nagano, T.; Hanaoka, K.; Suzuki, T.; Miyata, N.; Nakagawa, H. Synthesis of a photocontrollable hydrogen sulfide donor using ketoprofenate photocages. *Chem. Commun.* 2014, 50, 587–589. [CrossRef]

82. Xiao, Z.; Bonnard, T.; Shakouri-Motlagh, A.; Wylie, R.A.L.; Collins, J.; White, J.; Heath, D.E.; Hagemeyer, C.E.; Connal, L.A. Arylthioamides as H2S Donors: L-Cysteine-Activated Releasing Properties and Vascular Effects in Vitro and in Vivo. *ACS Med. Chem. Lett.* 2013, 4, 904–908. [CrossRef]

83. Foster, J.C.; Powell, C.R.; Radzinski, S.C.; Matson, J.B. S-aroylthiooximes: A facile route to hydrogen sulfide releasing compounds with structure-dependent release kinetics. *Org. Lett.* 2014, 16, 1558–1561. [CrossRef]

84. Devarie-Baez, N.O.; Bagdon, P.E.; Peng, B.; Zhao, Y.; Park, C.M.; Xian, M. Light-induced hydrogen sulfide release from “caged” gem-dithiols. *Org. Lett.* 2013, 15, 2786–2789. [CrossRef]

85. Fukushima, N.; Ieda, N.; Sasakura, K.; Nagano, T.; Hanaoka, K.; Suzuki, T.; Miyata, N.; Nakagawa, H. Synthesis of a photocontrollable hydrogen sulfide donor using ketoprofenate photocages. *Chem. Commun.* 2014, 50, 587–589. [CrossRef]

86. Xiao, Z.; Bonnard, T.; Shakouri-Motlagh, A.; Wylie, R.A.L.; Collins, J.; White, J.; Heath, D.E.; Hagemeyer, C.E.; Connal, L.A. Triggered and Tunable Hydrogen Sulfide Release from Photogenerated Thiobenzaldehydes. *Chemistry 2017*, 23, 11294–11308. [CrossRef]

87. Zheng, Y.; Yu, B.; Ji, K.; Pan, Z.; Chittavong, V.; Wang, B. Esterase-Sensitive Prodrugs with Tunable Release Rates and Direct Generation of Hydrogen Sulfide. *Angew. Chem. (Int. Ed. Engl.)* 2016, 55, 4514–4518. [CrossRef]

88. Kang, J.; Li, Z.; Organ, C.L.; Park, C.M.; Yang, C.T.; Pacheco, A.; Peng, B.; Lefer, D.J.; Xian, M. pH-Controlled Hydrogen Sulfide Release for Myocardial Ischemia-Reperfusion Injury. *J. Am. Chem. Soc.* 2016, 138, 6336–6339. [CrossRef]

89. Steiger, A.K.; Zhao, Y.; Thum, M.D. Emerging Roles of Carbonyl Sulfide in Chemical Biology: Sulfide Transporter or Gasotransmitter? *Antioxid. Redox Signal.* 2018, 28, 1516–1532. [CrossRef]

90. Steiger, A.K.; Pardue, S.; Kevil, C.G.; Thum, M.D. Self-Immobilating Thiocarbamates Provide Access to Triggered H2S Donors and Analyte Replacement Fluorescent Probes. *J. Am. Chem. Soc.* 2016, 138, 7256–7259. [CrossRef]

91. Xu, W.; Cui, C.; Cui, C.; Chen, Z.; Zhang, H.; Cui, Q.; Xu, G.; Fan, J.; Han, Y.; Tang, L.; et al. Hepatocellular cystathionine gamma lyase/hydrogen sulfide attenuates nonalcoholic fatty liver disease by activating farnesoid X receptor. *Hepatology 2022*. [CrossRef]
93. Chen, L.; Gao, Y.; Zhao, Y.; Yang, G.; Wang, C.; Zhao, Z.; Li, S. Chondroitin sulfate stimulates the secretion of H2S by Desulfovibrio to improve insulin sensitivity in NAFLD mice. *Int. J. Biol. Macromol.* 2022, 213, 631–638. [CrossRef]

94. Werge, M.P.; McCann, A.; Galsgaard, E.D.; Holst, D.; Bugge, A.; Albrechtsen, N.J.W.; Gluud, L.L. The Role of the Transsulfuration Pathway in Non-Alcoholic Fatty Liver Disease. *J. Clin. Med.* 2021, 10, 1081. [CrossRef]

95. Liu, Z.; Liu, M.; Fan, M.; Pan, S.; Li, S.; Chen, M.; Wang, H. Metabolomic-proteomic combination analysis reveals the targets and molecular pathways associated with hydrogen sulfide alleviating NAFLD. *Life Sci.* 2021, 264, 118629. [CrossRef]

96. Sun, H.J.; Wu, Z.Y.; Nie, X.W.; Wang, X.Y.; Bian, J.S. Implications of hydrogen sulfide in liver pathophysiology: Mechanistic insights and therapeutic potential. *J. Adv. Res.* 2021, 27, 127–135. [CrossRef]

97. Luo, Z.L.; Tang, L.J.; Wang, T.; Dai, R.W.; Ren, J.D.; Cheng, L.; Xiang, K.; Tian, F.Z. Effects of treatment with hydrogen sulfide on methionine-choline deficient diet-induced non-alcoholic steatohepatitis in rats. *J. Gastroenterol. Hepatol.* 2014, 29, 215–222. [CrossRef]

98. Sarna, L.K.; Sid, V.; Wang, P.; Siow, Y.L.; House, J.D.; Karmin, O. Tyrosol Attenuates High Fat Diet-Induced Hepatic Oxidative Stress: Potential Involvement of Cystathionine beta-Synthase and Cystathionine gamma-Lyase. *Lipids* 2016, 51, 583–590. [CrossRef]

99. Zhang, J.; Shi, C.; Wang, H.; Gao, C.; Chang, P.; Chen, X.; Shan, H.; Zhang, M.; Tao, L. Hydrogen sulfide protects against cell damage through modulation of PI3K/Akt/Nrf2 signaling. *Int. J. Biochem. Cell Biol.* 2019, 117, 105636. [CrossRef]

100. Wallace, J.L.; Wang, R. Hydrogen sulfide-based therapeutics: Exploiting a unique but ubiquitous gasotransmitter. *PLoS ONE* 2015, 10, e0125779. [CrossRef] [PubMed]

101. Sun, L.; Zhang, S.; Yu, C.; Pan, Z.; Liu, Y.; Zhao, J.; Wang, X.; Yun, F.; Zhao, H.; Yan, S.; et al. Hydrogen sulfide reduces serum triglyceride by activating liver autophagy via the AMPK-mTOR pathway. *Am. J. Physiol. Endocrinol. Metab.* 2018, 309, E925–E935. [CrossRef] [PubMed]

102. Yang, F.; Zhang, L.; Gao, Z.; Sun, X.; Yu, M.; Dong, S.; Wu, J.; Zhao, Y.; Xu, C.; Zhang, W.; et al. Exogenous H2S Protects Against Diabetic Cardiomyopathy by Activating Autophagy via the AMPK/mTOR Pathway. *Cell. Physiol. Biochem.* 2017, 43, 1168–1187. [CrossRef] [PubMed]

103. Ji, L.; Li, L.; Qu, F.; Zhang, G.; Wang, Y.; Bai, X.; Pan, S.; Xue, D.; Wang, G.; Sun, B. Hydrogen sulfide exacerbates acute pancreatitis by over-activating autophagy via AMPK/mTOR pathway. *J. Cell. Mol. Med.* 2016, 20, 2349–2361. [CrossRef]

104. Wu, Y.C.; Wang, X.J.; Yu, L.; Chan, F.K.; Cheng, A.S.; Yu, J.; Sung, J.J.; Wu, W.K.; Cho, C.H. Hydrogen sulfide lowers proliferation and induces protective autophagy in colon epithelial cells. *PLoS ONE* 2012, 7, e37572. [CrossRef]

105. Zhao, H.; Liu, H.; Yang, Y.; Lan, T.; Wang, H.; Wu, D. Hydrogen Sulfide Plays an Important Role by Regulating Endoplasmic Reticulum Stress in Diabetes-Related Diseases. *Int. J. Mol. Sci.* 2022, 23, 7170. [CrossRef]

106. Iqbal, I.K.; Bajeli, S.; Sahu, S.; Bhat, S.A.; Kumar, A. Hydrogen sulfide-induced GAPDH sulfhydration disrupts the CCAR2-SIRT1 interaction to initiate autophagy. *Autophagy* 2021, 17, 3511–3529. [CrossRef]

107. Wang, S.S.; Chen, Y.H.; Chen, N.; Wang, L.J.; Chen, D.X.; Dooley, S.; Ding, H.G. Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway. *Cell Death Dis.* 2017, 8, e2688. [CrossRef]

108. Chen, X.; Zhao, X.; Cai, H.; Sun, H.; Hu, Y.; Huang, X.; Kong, W.; Kong, W. The role of sodium hydrosulfide in attenuating the aging process via PI3K/AKT and CaMKKbeta/AMPK pathways. *Redox Biol.* 2017, 12, 987–1003. [CrossRef]

109. Wang, J.; Wu, D.; Wang, H. Hydrogen sulfide protects spinal cord ischemia-reperfusion injury by regulating endoplasmic reticulum stress-induced autophagy in mice. *Physiol. Res.* 2019, 68, 335–345. [CrossRef] [PubMed]

110. Li, L.; Jiang, H.K.; Li, Y.P.; Guo, Y.P. Hydrogen sulfide protects spinal cord and induces autophagy via miR-30c in a rat model of spinal cord ischemia-reperfusion injury. *J. Biomed. Sci.* 2015, 22, 50. [CrossRef]

111. Zhang, H.; Guo, C.; Wu, D.; Zhang, A.; Gu, T.; Wang, L.; Wang, C. Hydrogen sulfide inhibits the development of atherosclerosis with suppressing CX3CR1 and CX3CL1 expression. *PLoS ONE* 2012, 7, e41147. [CrossRef]

112. Sutti, S.; Locatelli, I.; Bruzzi, S.; Jindal, A.; Vacciano, M.; Bazzola, C.; Albano, E. CX3CR1-expressing inflammatory dendritic cells contribute to the progression of atherosclerosis. *Clin. Sci.* 2015, 129, 797–808. [CrossRef] [PubMed]

113. Chi, Q.; Wang, D.; Hu, X.; Li, S.; Li, S. Hydrogen Sulfide Gas Exposure Induces Necroptosis and Promotes Inflammation through the MAPK/NF-kappaB Pathway in Broiler Spleen. *Oxidative Med. Cell. Longev.* 2019, 2019, 8061823. [CrossRef]

114. 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. [CrossRef] [PubMed]

115. Wu, D.D.; Wang, D.Y.; Li, H.M.; Guo, J.C.; Duan, S.F.; Ji, X.Y. Hydrogen Sulfide as a Novel Regulatory Factor in Liver Health and Disease. *Oxid. Med. Cell. Longev.* 2019, 2019, 3831713. [CrossRef]

116. Koike, S.; Ogawara, Y.; Shibuya, N.; Kimura, H.; Ishii, K. Polysulfide exerts a protective effect against cytotoxicity caused by t-bathyldihydropyrene in neuroblastoma cells. *FEBS Lett.* 2013, 587, 3548–3555. [CrossRef]

117. Yang, G.; Zhao, K.; Ju, Y.; Mani, S.; Cao, Q.; Putikala, S.; Kheraper, N.; Wu, L.; Wang, R. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. *Antioxid. Redox Signal.* 2013, 18, 1906–1919. [CrossRef] [PubMed]

118. Cai, J.; Shi, X.; Wang, H.; Fan, J.; Feng, Y.; Lin, X.; Yang, J.; Cui, Q.; Tang, C.; Xu, G.; 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. Et Biophys. Acta* 2016, 1861, 419–429. [CrossRef]
119. Tailleux, A.; Wouters, K.; Staels, B. Roles of PPARs in NAFLD: Potential therapeutic targets. *Biochim. Et Biophys. Acta* 2012, 1821, 809–818. [CrossRef]

120. Suppli, M.P.; Rigbolt, K.T.G.; Veidal, S.S.; Heebøll, S.; Eriksen, P.L. Hepatic transcriptome signatures in patients with varying degrees of nonalcoholic fatty liver disease compared with healthy normal-weight individuals. *American Journal of Physiology Gastrointest. Liver Physiol.* 2019, 316, G462–G472. [CrossRef]

121. Zhang, X.; Bian, J.S. Hydrogen sulfide: A neuromodulator and neuroprotectant in the central nervous system. *ACS Chem. Neurosci.* 2014, 5, 876–883. [CrossRef]

122. Krishnan, N.; Fu, C.; Pappin, D.J.; Tonks, N.K. H2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Signal.* 2011, 4, ra86. [CrossRef] [PubMed]

123. Yadav, V.; Gao, X.H.; Willard, B.; Hatzoglou, M.; Banerjee, R.; Kabil, O. Hydrogen sulfide modulates eukaryotic translation initiation factor 2α (eIF2α) phosphorylation status in the integrated stress-response pathway. *J. Biol. Chem.* 2017, 292, 13143–13153. [CrossRef]

124. Zhang, D.; Du, J.; Tang, C.; Huang, Y.; Jin, H. H(2)S-Induced Sulfhydration: Biological Function and Detection Methodology. *Front. Pharmacol.* 2017, 8, 608. [CrossRef]

125. Wu, D.; Zheng, N.; Qi, K.; Cheng, H.; Sun, Z.; Gao, B.; Zhang, Y.; Pang, W.; Huangfu, C.; Ji, S.; 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. [CrossRef]