Review

Disrupted H₂S Signaling by Cigarette Smoking and Alcohol Drinking: Evidence from Cellular, Animal, and Clinical Studies

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Abstract: The role of endogenous hydrogen sulfide (H₂S) as an antioxidant regulator has sparked interest in its function within inflammatory diseases. Cigarette and alcohol use are major causes of premature death, resulting from chronic oxidative stress and subsequent tissue damage. The activation of the Nrf2 antioxidant response by H₂S suggests that this novel gasotransmitter may function to prevent or potentially reverse disease progression caused by cigarette smoking or alcohol use. The purpose of this study is to review the interrelationship between H₂S signaling and cigarette smoking or alcohol drinking. Based on the databases of cellular, animal, and clinical studies from Pubmed using the keywords of H₂S, smoking, and/or alcohol, this review article provides a comprehensive insight into disrupted H₂S signaling by alcohol drinking and cigarette smoking-caused disorders. Major signaling and metabolic pathways involved in H₂S-derived antioxidant and anti-inflammatory responses are further reviewed. H₂S supplementation may prove to be an invaluable asset in treating or preventing diseases in those suffering from cigarette or alcohol addiction.

Keywords: H₂S; cigarette smoking; alcohol drinking; oxidative stress; inflammation

1. Introduction

Cigarette smoking and alcohol consumption are the leading causes of the global burden of disease and are major contributors to premature mortality in industrial countries. Cigarette smoking and alcohol drinking can cause damage in nearly all body organs, especially cardiovascular, nervous, hepatic, gastrointestinal and pulmonary systems. Systemic disease caused by cigarettes or alcohol use can be acute or chronic [1,2]. Chronic obstructive pulmonary disease (COPD), alcoholic fatty liver disease, cardiomyopathy, neurodegeneration, gastric ulceration, atherosclerosis, fetal alcohol spectrum disorder, low birth weight, and many forms of cancer are all can be caused by cigarette smoking, alcohol drinking, or both [3–6]. Concurrent consumption of alcohol and cigarette smoking further increase the risk of developing these diseases [7]. Treatment of these diseases has been progressing, with some focusing on ameliorating symptoms in late stage disease, but the most effective treatment is abstinence from using cigarettes and alcohol [8,9].

The pathology of cigarette smoking and alcohol drinking-caused diseases is generally attributed to excessive oxidative stress and chronic inflammation [10,11]. The primary effector of disease is oxidative imbalance caused by the rapid accumulation of reactive oxygen species (ROS) within the cells. Cigarettes introduce ROS via a multitude of cytotoxic compounds, including nicotine, aldehydes, heavy metals, polycyclic aromatic hydrocarbons, and more, while alcohol introduces oxidative stress through the metabolism of ethanol to acetaldehyde [12,13]. High level of oxidative stress triggers NFκB-mediated release of proinflammatory cytokines, such as TNF-α and interleukins, which consequently recruit the immune system to the affected tissues [14–16]. Recruited neutrophils induce further

oxidative stress through the production of ROS, and the secretion of matrix metalloproteases for degradation of extracellular matrix [17,18]. Constitutive oxidative stress results in cell death, and the repeated destruction and reconstruction of the extracellular matrix eventually leading to fibrosis. These effects cumulatively induce organ dysfunction and tissue death (Figure 1).
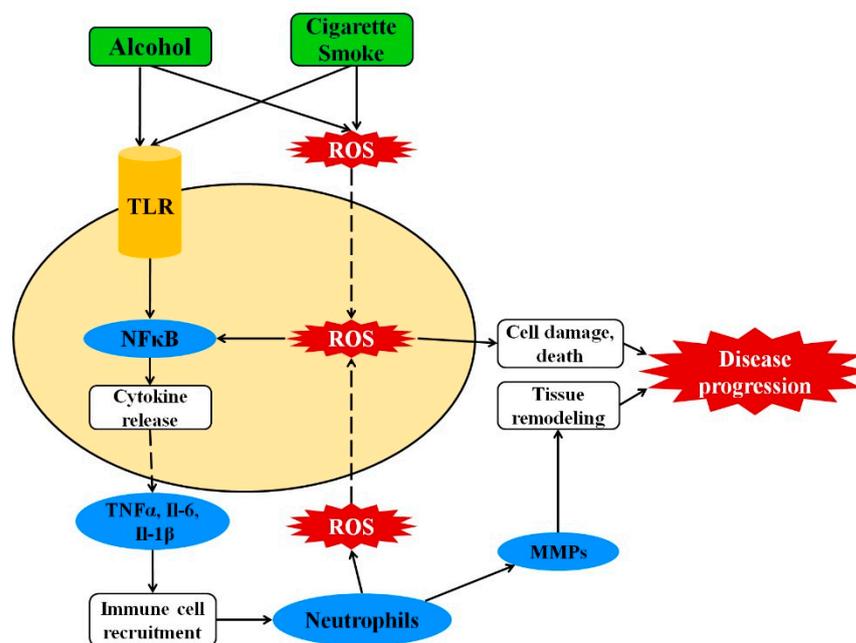
Figure 1. Cigarette smoking and alcohol drinking induce the oxidative and inflammatory response. Exposure to cigarette or alcohol leads to oxidative stress and stimulation of NFκB. NFκB promotes secretion of proinflammatory cytokines TNFα, IL-6, and IL-1β, which then strengthen immune cell recruitment. Recruited neutrophils produce additional ROS and release metalloproteases, which induce cell damage and matrix degradation, respectively. The net result of constitutive activation of inflammatory pathways is tissue destruction and disease progression. Abbreviations: NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; TLR, toll-like receptor; TNFα, tumor necrosis factor α; IL, interleukin; ROS, reactive oxygen species.
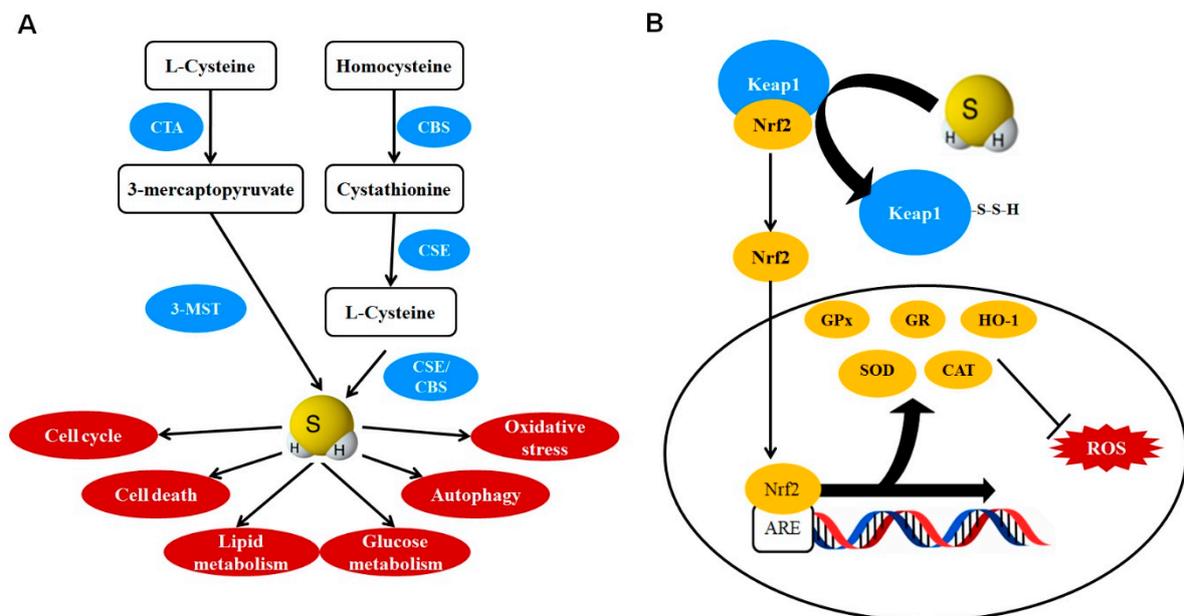
2. H$_2$S as a Novel Gasotransmitter

Instantly recognizable by its distinct rotten-egg smell, hydrogen sulfide (H$_2$S) has traditionally been viewed as nothing more than an unpleasant smelling and toxic gas. At high concentrations this certainly holds true, as H$_2$S prevents cellular respiration by binding with and inhibiting cytochrome C oxidase [19]. During the past decades, however, a different story has emerged. First discovered as an endogenous molecule in the 1960s, the gaseous compound was initially believed to be a metabolic waste product. It was not until the mid-1990s that a physiological role for the so-called waste was uncovered, when it was found that H$_2$S activates long-term potentiation in the brain [20]. Since then, H$_2$S has been classed as the third gasotransmitter, alongside carbon monoxide and nitric oxide, and a novel field of research has developed around the diverse biological effects of H$_2$S [21,22].

The endogenous production of H$_2$S is well regulated, and at least three distinct enzymatic pathways contribute to endogenous production of H$_2$S in various tissues. The three pathways are denoted by the primary enzyme involved, those being cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptosulfur transferase (3MST) (Figure 2A) [23,24]. CBS is the most prominent within the brain, while CSE plays the largest role in the other major tissues and 3MST plays a secondary or tertiary role in most tissues [23].

The physiological functions of H$_2$S include regulation of cellular senescence, cell cycle, metabolism, vasorelaxation, autophagy, and oxidative stress (Figure 2A) [25–27]. H$_2$S

post-translational modification of protein by S-sulfhydration mediates most of the cellular functions [28,29]. S-sulfhydration consists of the formation of a persulfide group (-SSH) or polysulfide chains on active cysteine residues within a protein [30,31]. These changes result in structural and conformational alterations, eventually changing protein function and catalytic ability [32]. The ubiquity of cysteine residues in proteins, ranging from structural disulfide bridges to catalytic sites, ensures that H_2S has wide ranging effects on many proteins [28,33]. There is some doubt on the direct formation of S-sulfhydration in free cysteine by H_2S [28]. Sulfane sulfur, a group of sulfur-containing compounds, may interact with oxidized cysteine in target protein resulting in formation of S-sulfhydration [29,34]. The generation of cysteine S-sulfhydration by sulfane sulfur depend on the location and oxidative status of individual cysteine residues [28,34]. S-sulfhydration is not only a post-translational modification of protein. Akaike et al. proved that cysteine S-sulfhydration can also occur at the translational stage with the aid of cysteinyl tRNA synthetase [35]. In contrast, thioredoxin 1, an important reducing enzyme that cleaves disulfides in proteins, facilitates protein S-desulfhydration [36]. Future studies need to explore the regulatory mechanisms of cysteine S-sulfhydration formation and map the global protein S-sulfhydrome in both health and disease.


Figure 2. H_2S is recognized as a novel gasotransmitter. (**A**) Enzymatic H_2S production mediated by CSE, CBS, and 3MST/CAT. Endogenously produced H_2S regulates diverse cellular functions, including cell cycle, cell death, lipid metabolism, glucose metabolism, oxidative stress, and autophagy. (**B**) H_2S mediates its antioxidant effects through activation of Keap1/Nrf2. S-sulfhydration of cysteine residues on Keap1 causes the release of Nrf2, which is then able to translocate into the nucleus and bind with ARE. Transcription of ARE leads to production of several AOEs, which collectively reduce oxidative stress. Abbreviations: 3-MST, 3-mercaptopyruvate sulfurtransferase; ARE, antioxidant response element; AOE, antioxidant enzyme; CAT, catalase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; CTA, cysteine transaminase; GPx, glutathione peroxidase; GR, glutathione reductase; H_2S, hydrogen sulfide; Keap1, Kelch-like ECH associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase; ROS, reactive oxygen species.

The most notable target for S-sulfhydration is the regulation of the Nrf2/Keap1 system. Nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2) is a master regulator of the antioxidant response, which translocates to the nucleus and binds with the antioxidant response element (ARE) [37]. Increased ARE transcription stimulates several antioxidant enzymes (AOEs), including superoxide dismutase, glutathione peroxidase, catalase, and heme oxygenase-1 [38]. These enzymes collectively act to maintain oxidative homeostasis

and reduce ROS-mediated damage within the cells. In the cytoplasm, Nrf2 is bound by Kelch-like ECH-associated protein 1 (Keap1), which promotes the ubiquitination and degradation of Nrf2 by the proteasome [37]. S-sulfhydation of Keap1 prevents binding to Nrf2, allowing for Nrf2 nuclear localization and AOE transcription, thus making H_2S as an activator of the Nrf2 antioxidant master switch (Figure 2B) [27]. In addition to increasing transcription of AOE, H_2S can directly reduce ROS levels by restoring glutathione levels and/or through chemical reduction of offending molecules [39,40].

Endogenous H_2S likely play a major role in the innate defense against oxidative stress and inflammation-mediated disease (Figure 3) [41]. Endogenous H_2S is influenced by many factors, including genetic, dietary, and environmental components. Major dietary sources of sulphur include cruciferous vegetables, such as broccoli or kale, and garlic. These foods contain many organosulphur compounds, which are readily metabolized by the body to yield H_2S, and are often considered H_2S donors as a result [42]. Regular exercise boosts endogenous H_2S production [43], while "unhealthy" habits such as high-fat diets and sedentary lifestyle decrease H_2S level due to lower expressions of H_2S-generating genes [44].
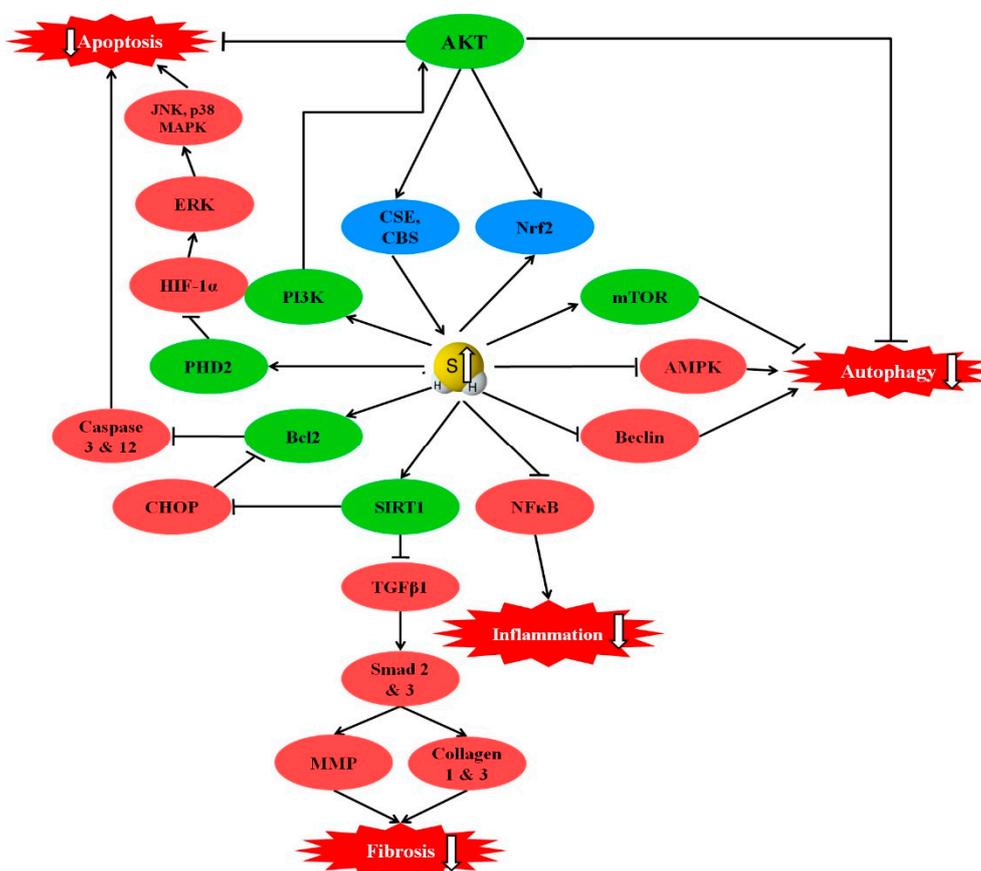
Figure 3. The signaling pathways underlying H_2S regulation of inflammation, apoptosis, fibrosis, and autophagy. Abbreviations: Bcl2, B-cell lymphoma 2; CHOP, C/EBP homologous protein; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; ERK, extracellular signal-regulated kinase; HIF-1α, hypoxia-inducible factor 1-alpha; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metallopeptidase; mTOR, mammalian target of rapamycin; TGFβ1, transforming growth factor beta 1.

Despite a large amount of H_2S research in both health and disease having been published, a comprehensive review on the relationship between H_2S signaling and smoking or drinking is lacking. Given the importance of H_2S in biology and medicine, this review article summarizes the recent research progress on the involvement of H_2S signaling in cell damage and organ dysfunction related to cigarette smoking and alcohol drinking.

Based on the databases from Pubmed, "H_2S and smoking", "H_2S and cigarettes", "H_2S and tobacco", "H_2S and alcohol", and "H_2S and ethanol" were used as phrases to search literature related to H_2S signaling in biology and medicine. The publications related to environmental pollution or H_2S toxicology were excluded from this review.

3. H_2S and Cigarette Smoking

3.1. Clinical Observations

Definitive links between smoking and the changes in H_2S levels in human have been uncovered. Serum H_2S level was significantly lower in smokers than nonsmokers [45]. The expression of CSE was decreased in smokers' lung tissue as detected by immunohistochemistry and Western blotting [46]. Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease and can be caused by long-term exposure to cigarette smoking. Serum H_2S is elevated in the patients with stable COPD, yet is lower in control smokers and those suffering from acute exacerbation of COPD (AE-COPD) [46]. In contrast, sputum H_2S levels in AE-COPD patients were higher than those with stable COPD [47]. It was further found that exhaled H_2S levels were similar in patients with AE-COPD, stable COPD, and healthy controls, while COPD patients without eosinophilia had significantly higher levels of exhaled H_2S compared with subjects with eosinophilia [48,49]. These findings point that disrupted H_2S signaling might be valuable marker to examine the different status of COPD. More excitingly, after undergoing a 10-day cycle of sulphurous thermal water inhalation, a reduction of the inflammatory activity was observed in a large number of heavy current and former smokers, suggesting the potential benefits of H_2S for smokers [50]. Moreover, Bates et al. provided some epidemiologic evidence that ambient H_2S exposures may benefit lung function, possibly through airway smooth muscle relaxation [51]. Maternal smoking during pregnancy increases the risk of asthma and borderline personality disorder-like symptoms in the progeny. A recent study further found that the expressions of three H_2S-generating genes, including CSE, CBS, and 3MST, were all lower in the placentas from mothers who smoked during pregnancy in comparison with the mothers who never smoked. Quitting smoking during the first trimester of pregnancy could partially improve the expressions of these H_2S-generating genes in the placentas [52]. More studies need to be conducted to validate if the downregulated expressions of these H_2S-generatiang enzymes might be a biomarker for asthma susceptibility in children.

3.2. Animal Studies

Exposure of laboratory animals to cigarette smoking can reproduce some morphologic and physiologic manifestations of diseases as happened in humans. Several rodent models have been employed in the study of H_2S signaling and cigarette smoking-induced organ damage. The results obtained in these preclinical animal studies demonstrated that H_2S signaling was often disrupted by cigarette smoking exposure, while exogenously applied H_2S was able to provide beneficial effects against cigarette smoking exposure-caused pulmonary and vascular damage, as well as abnormal development of natal offspring (Table 1).

3.2.1. H_2S Protects from Maternal/Prenatal Cigarette Smoking Exposure-Induced Damage

Maternal cigarette smoking exposure has been found to increase oxidative stress, mitochondrial dysfunction, and cell apoptosis in offspring. Zhang et al. first observed that cigarette smoking exposure to rats during pregnancy blunted hypercapnic respiratory responses in the neonates, which could be relieved by H_2S pretreatment [53]. The same group further reported that H_2S protected the offspring from apoptosis and helped the recovery of central chemoreception via the activation of mitoK$_{ATP}$ channels [54]. It was proposed that H_2S may have potential therapeutic value for maternal cigarette smoking exposure-induced central chemoreception deficit-related diseases, such as sudden infant death syndrome.

Prenatal cigarette smoking exposure is known to induce respiratory problems in infants. The mRNA and protein expression of 3MST was upregulated in the neurons of medullary respiratory nuclei of neonatal rats by intrauterine cigarette smoking exposure, indicating that endogenous H_2S derived from 3MST may protect medullary respiratory centers against cigarette smoking-caused injury [55]. Wang et al. found that cigarette smoking exposure caused a more slowly and deeply breathing in rat neonates under sevoflurane anesthesia, while pretreatment with H_2S via intraperitoneal injection NaHS alleviated sevoflurane-induced respiratory suppression [56]. Mechanistically, H_2S was also able to prevent the increase in the malondialdehyde level, upregulation of inflammatory response, and suppression of antioxidase activity in the neonatal rats induced by prenatal cigarette smoking exposure [57,58]. H_2S was also able to improve cigarette smoking-caused fetal and placental development. In an animal model of cigarette smoking exposure in pregnant rats, the administration of NaHS normalized the reduced fetal and placental weights by cigarette smoking [59]. Further studies demonstrated that H_2S relieved cigarette smoking-induced abnormalities of junctional and labyrinthine zones, and ultrastructural alterations in rat placenta [60].

In another study, mice were exposed to secondhand cigarette smoking throughout the gestational period, and then H_2S biogenesis in lungs from the pups of the F1 and F2 generation were analyzed. It was found that mouse lung exposed gestationally to cigarette smoking had significantly lower levels of CSE, CBS, and 3MST in both the F1 and F2 generation. Moreover, the lungs from F1 and F2 progenies displayed with impaired angiogenesis and alveolarization, suggesting that a lower level of H_2S is correlated with the increased risk of lung disorders [52].

3.2.2. H_2S Attenuates Cigarette Smoking-Induced Lung Damage

Long-term cigarette smoking exposure can induce airway remodeling, thus damaging the respiratory function. By using a mouse model of cigarette smoking exposure, Guan et al. found that treatment with NaHS significantly attenuated airway thickening and collagen deposition, as characterized by decreased expressions of α-SMA and collagens and myofibroblast accumulation [61]. The protective role of H_2S against airway remodeling was attributed to its inhibition of SIRT1-mediated TGF-β1/Smad3 activation and epithelial–mesenchymal transition. Han et al. also reported that NaHS ameliorated cigarette smoking-induced thickness of bronchial walls and emphysema development in mice via AKT/Nrf2 pathways [62]. With a rat model of cigarette smoking exposure, Wang et al. demonstrated that H_2S inhibited cigarette smoking-caused oxidative stress, inflammation, and airway remodeling through inhibition of the TGF-β1/Smad pathway [63].

CSE deficiency abolished endogenous H_2S generation in mouse lung and promoted the secretion of cytokines and chemokines in bronchoalveolar lavage fluid following cigarette smoking exposure and respiratory syncytial virus infection [64]. These data suggest that the lower level of endogenous H_2S due to immature H_2S-genarating enzymes could cause more severe manifestations of virus infection under cigarette smoking exposure or other stress conditions. In one study, blockage of endogenous H_2S generation by propargylglycine (PPG), a CSE inhibitor, however, did not worsen the detrimental effect of cigarette smoking exposure on pulmonary remodeling, excluding the involvement of CSE-derived H_2S in lung protection [65]. In contrast, other studies demonstrated that PPG aggravated lung pathology score and increased airway reactivity in cigarette smoking-exposed rats, suggesting that endogenous H_2S may have a protective role of anti-inflammation and bronchodilation in chronic cigarette smoking-induced pulmonary injury [66–68]. The discrepancy may be due to the dose and time of cigarette smoking exposure and PPG administration, as well as the age of rats used in these studies.

3.2.3. H_2S Ameliorates Cigarette Smoking Exposure-Induced Cardiac Damage

Smoking is a risk factor causing cardiovascular disease, and about a third of smoking-related deaths result from heart complications. Animal models of cigarette smoking

exposure all demonstrated the potential protection of H_2S against abnormal heart functions. In a rat model of cigarette smoking exposure, CSE expression was downregulated in heart tissues and H_2S levels in plasma were also lower, and the left ventricular systolic function was remarkably lower. Supplementation of exogenous H_2S would improve the heart functions. The protective role of H_2S against cigarette smoking -induced cardiac dysfunction was associated with PI3K/Akt-mediated Nrf2 nuclear translocation and increased expression of antioxidant genes followed by reduced cell apoptosis and autophagy [69,70]. Consistent with these studies, Wiliński et al. also proved that administration of nicotine to mice caused a marked decrease in H_2S level in heart tissues [71]. In the presence of vitamin D3, nicotine-induced structural disorder in the aortic and carotid arterial wall could be ameliorated by exogenous applied H_2S [72]. In addition, cigarette smoking exposure lowered CSE.

Table 1. Animal models used for analyzing H_2S signaling and cigarette smoking-induced damage.

Animal Species	Cigarette Smoking (CS) Administration	H_2S Supplement	Organ Damage	Target Signaling Pathway	Reference
BALB/c mice	6 h/day, 1.52 mg/m^3 during gestation	N/A	Asthma, bronchopulmonary dysplasia	CSE/CBS, TGFβ1, SOX2	Singh et al. [52]
Sprague–Dawley rats	10 cigarettes/h, twice daily, gestation day 7–20	56 µmole/kg NaHS, daily, 30 min before CS, i.p.	Brainstem, respiratory dysfunction	N/A	Zhang et al. [53]
Sprague–Dawley rats	10 cigarettes/h, twice daily, gestation day 1–20	56 µmole/kg NaHS, daily, 30 min before CS, i.p.	Hypoglossal nerve damage, chemoreception impairment	Caspase/Bax	Lei et al. [54]
Sprague–Dawley rats	10 cigarettes/h, twice daily, gestation day 7–20	56 µmole/kg NaHS, daily, 30 min before CS, i.p.	Decreased respiratory function	N/A	Wang et al. [56]
Sprague–Dawley rats	10 cigarettes/h, twice daily, gestation day 7–20	56 µmole/kg NaHS, daily, 30 min before CS, i.p.	Excitatory synapse disorder, respiratory misregulation	Phox2b, AOE/ROS	Yan et al. [57]
Sprague–Dawley rats	10 cigarettes/h, twice daily, gestation day 7–20	56 µmole/kg NaHS, daily, 30 min before CS, i.p.	Medulla oblongata and hypoglossal nerve damage	AOE, TNFα, IL-6/1β	Yan et al. [58]
C57BL/6J mice	9 filter-tipped cigarettes for 2 h twice daily, 6 days/week	40 mg/kg NaHS via atomization inhalation for 30 min, twice daily	COPD	S1RT/ TGFβ1, AOE/ROS	Guan et al. [61]
C57BL/6 mice	1 puff/min and 10 puffs/cigarette for 1 h daily, 5 days per week for 12 or 24 weeks	50 µmole/kg NaHS, daily, 30 min before CS, i.p.	Emphysema, bronchial remodeling, pulmonary vascular remodeling	TNFα, Nrf2, p-AKT, Caspase	Han et al. [62]

Table 1. *Cont.*

Animal Species	Cigarette Smoking (CS) Administration	H$_2$S Supplement	Organ Damage	Target Signaling Pathway	Reference
Sprague–Dawley rats	20 cigarettes for 2 h, twice daily, 6 days per week	40 ppm H$_2$S exposure, 8 h daily for seven days	COPD	ROS, TNFα/IL6, TGFβ1/Smad	Wang et al. [63]
Sprague–Dawley rats	20 cigarettes for 4 h, 7 days per week	14 μmole/kg NaHS, daily, 30 min before CS, i.p.	COPD, reduced pulmonary response	CSE, TNFα/IL6	Chen et al. [68]
Sprague–Dawley rats	10 cigarettes for 30 min, 4 times daily	14 μmole/kg NaHS, daily, 30 min before CS, i.p.	Left ventricle structure and function damage	CSE/Nrf2, AOE/ROS, PI3K/GS3Kβ	Zhou et al. [69]
Sprague–Dawley rats	10 cigarettes for 30 min, 4 times daily	8 μmole/kg NaHS, daily, i.g.	Left ventricle structure and function damage	Caspase/Bax, JNK/p38, PI3K, Beclin-1, AMPK/mTOR	Zhou et al. [70]
CBA mice	1.5 mg/kg nicotine, daily, i.p.	N/A	Reduced H$_2$S production in heart/kidney	N/A	Wiliński et al. [71]
Sprague–Dawley rats	25 mg/kg nicotine, daily, i.g.	56 μmole/kg NaHS, daily, i.p.	Calcification of carotid and aorta	CHOP/CAS, Calponin/SM22α	Li et al. [72]
Sprague–Dawley rats	20 cigarettes, daily, for 30 days	N/A	Vascular constriction	CSE, SUR-2, K$_{ATP}$	Zhang et al. [73]

Expression in rat thoracic aorta and inhibited aortic vascular relaxation, which could in part explain the association between cigarette smoking and disrupted heart functions [73]. Given this evidence, it could be feasible for targeting H$_2$S signaling for the prevention and therapy of cigarette smoking-induced vascular disorders.

3.3. Cellular Studies

Alveolar epithelial cells in lung are the direct targets for damage by smoking. Exposure to cigarette smoking in human alveolar basal epithelial cells (A549) leads to lower cell viability, higher oxidative stress and cell apoptosis, and increased levels of inflammatory factors, all of which could be attenuated by the addition of NaHS. It was further demonstrated that H$_2$S upregulated SIRT1 and protected cigarette smoking-induced mitochondrial dysfunctions in epithelial cells [74]. The following study from the same group showed that H$_2$S was also able to inhibit cigarette smoking-induced activation of PHD2/HIF-1α signaling and ERK/JNK/p38 MAPK pathways in alveolar epithelial cells [75]. With bronchial epithelia cells, Lin et al. proved that H$_2$S inhibited nicotine-induced morphological changes of apoptosis via inhibition of endoplasmic reticulum stress [76]. These findings suggest that H$_2$S has potential therapeutic value in the treatment of cigarette smoking-caused lung damage by directly protecting against epithelial cell death.

The animal studies showed that H$_2$S was able to reduce cigarette smoking-induced pulmonary hypertension and vascular remodeling in mice [62]. By using pulmonary artery endothelial cells, Han et al. observed that H$_2$S protected against cigarette smoking-induced apoptosis and downregulation of Akt and Nrf2 [62]. The airway smooth muscle cells (ASMCs) are important for controlling airway structure and dilation. The loss of contractile phenotype of ASMCs by cigarette smoking leads to airway hyperresponsiveness and remodeling followed by the development of COPD. Perry et al. reported that H$_2$S inhibited proliferation and cytokine release in ASMCs isolated from nonsmokers and smokers, but had less effect on the cells from COPD patients [77]. This points to the role of H$_2$S in the stabilization of ASMC phenotype for a novel therapeutic avenue against COPD.

Many studies have reported that cigarette smoking is also a driving factor for periodontitis and bone loss [78,79]. Nicotine, one of the major constitutes in cigarettes, was reported to inhibit osteoblastic differentiation [78]. In contrast, H_2S was able to promote osteoblastic differentiation in a nicotine-incubated human periodontal ligament cell model via the MAPK/PKC/NF-κB pathways, suggesting the potential of H_2S against cigarette smoking-induced bone loss [78].

4. H$_2$S and Alcohol Consumption

4.1. Clinical Observations

Clinical evidence about the interaction of H_2S signaling and alcohol drinking in humans is lacking. One paper reported that H_2S levels were increased in the breath of chronic alcohol users, suggesting that circulating H_2S may be elevated by alcohol drinking [80]. More studies need to be investigated to explore whether H_2S signaling is altered in alcohol users.

4.2. Animal Studies

Animal models are often used in the laboratory to mimic different aspects of human alcohol consumption, including chronic ad libitum ethanol feeding through liquid diet and chronic/acute intragastric ethanol administration. The advances from these animal studies help for understanding the molecular and behavioral changes associated with human alcoholism. The accumulated evidence suggests that H_2S signaling often involves alcohol-caused organ damage (Table 2).

4.2.1. H$_2$S Protects from Alcohol-Induced Liver Damage

Many H_2S-releasing donors have been shown to inhibit alcohol-induced liver damage. In both mouse and rat models of alcohol exposure, the administration of diallyl trisulfide, diallyl disulfide, garlic oil, or garlic polysaccharide significantly limited lipid accumulation and reduced tissue damage [81–86]. These H_2S donors were also able to decrease circulating liver-damage markers, such as alanine aminotranferease and aspartate transaminase [83–85,87]. Aldehyde dehydrogenase is a rate-limiting enzyme for ethanol metabolism by changing highly toxic acetaldehyde to nontoxic acetate. Recent evidence suggests the enzymatic activity of aldehyde dehydrogenase could be inhibited in the rat liver by some sulfane sulfur species, such as garlic-derived allyl sulfides, which act as sources for releasing H_2S slowly in the body [88]. Similar to disulfiram, H_2S may be used for the treatment of alcohol abuse and alcohol dependence by promoting the accumulation of acetaldehyde in the body.

Table 2. Animal models used for analyzing H_2S signaling and alcohol-induced damage.

Animal Species	Alcohol Administration	H$_2$S Supplement	Organ Damage	Target Signaling Pathway	Reference
Kun–Ming mice	50% (*v/v*) ethanol, 12 mL/kg, 16 h before sacrifice	30 mg/kg diallyl trisulfide (DATS), orally, for 7 days prior to ethanol	Liver damage	AOE/ROS	Zeng et al. [82]
Sprague–Dawley rats	56% (*v/v*) ethanol, 10 mL/kg daily for 8 weeks, gavage	25–100 mg/kg DATS, daily, weeks 5–8	Alcohol fatty liver, steatosis	PPAR-α/SREBP-1c, AOE/ROS, Cas/Bcl2	Chen et al. [83]
Kun–Ming mice	5 g/kg ethanol at 12 h intervals, sacrificed 4 h after third dose, i.g.	25–100 mg/kg diallyl disulfide (DADS), daily, for 7days by gavage	Liver injury, hydropic degeneration	Nrf2/HO-1, ROS, Cas, MAPKs	Zeng et al. [84]

Table 2. *Cont.*

Animal Species	Alcohol Administration	H₂S Supplement	Organ Damage	Target Signaling Pathway	Reference
Kun–Ming mice	50% (*v/v*) ethanol, 12 mL/kg, sacrificed at 4, 8, and 16 h after exposure	50, 100, 200 mg/kg garlic oil, 2 h before ethanol exposure	Liver injury, steatosis	AOE/ROS, PPAR-α/SREBP-1, FAS, CYP2E1	Zeng et al. [85]
Kun–Ming mice	56% ethanol, 6 mL/kg, orally, for 30 days	150–250 mg/kg garlic power, daily 5 h after ethanol exposure	Liver fibrosis, steatosis	AOE/ROS, TNFα/TGFβ1	Wang et al. [86]
Sprague–Dawley rats	10–12 g/kg ethanol, daily, for 45 days i.g.	200 mg/kg DATS, daily, i.g.	Alcoholic steatohepatitis	CYP2E1, TNFα/Il-4, TGFβ1/Smad	Ronis et al. [87]
Kun–Ming mice	4% ethanol solution as sole source of drinking water freely for 12 weeks	50 μmole/kg NaHS, daily, i.p.	Alcoholic cardiomyopathy	PI3K/AKT, Beclin, TGFβ1/MMP	Liang et al. [89]
Wistar rats	10–30% alcohol per day by i.g for 18 weeks	30 μmole/l NaHS in drinking water	Left ventricular structure and function damage	AOE/ROS, Bax/Bcl2	Zhou et al. [90]
Sprague–Dawley rats	5 mL/kg ethanol, orally	25–50 mg/kg Tert-butylhydroquinone (tBHQ) for 10 days, orally	Gastric ulcers	ROS, COX2, NFkB, Nrf2/HO-1	Rahman et al. [91]
Swiss mice	50% ethanol, 0.5 mL/25 g, gavage, sacrificed 1 h after ethanol	Cysteine (25–100 mg/kg), NaHS (75–300 μmole), or propargylglycine (15–150 mg/kg), gavage, 30 min prior to ethanol	Gastric ulcers	K$_{ATP}$ channels and afferent neurons/TRPV1 receptors	Medeiros et al. [92]
C57BL/6 mice	50% ethanol, 2.5 mL/kg, gavage, sacrificed 1 h after ethanol	l-cysteine or d-cysteine (100 mg/kg) or propargylglycine (100 mg/kg), gavage, 30 min prior to ethanol	Gastric ulcers	d-amino acid oxidase	Souza et al. [93]
Swiss mice	50% ethanol, 0.5 mL/25 g, gavage, sacrificed 1 h after ethanol	150 μmole/kg NaHS; 27 μmole/kg Lawesson's reagent; 100 mg/kg cysteine; orally, 30 min prior to ethanol	Gastric ulcers	AMPK	de Araújo et al. [94]
Wistar rats	100% ethanol, 1 mL, by gavage, sacrificed at 2 h	15, 50 or 150 μM Na₂S 30 min prior to ethanol	Gastric ulcers	N/A	Velázquez-Moyado et al. [95]

Table 2. *Cont.*

Animal Species	Alcohol Administration	H2S Supplement	Organ Damage	Target Signaling Pathway	Reference
Wistar rats	100% ethanol, 0.2 mL, by gavage, sacrificed after 30 min	50 mg/kg cysteine, orally	Gastric ulcers	N/A	Velázquez-Moyado et al. [96]
Wistar rats	100% ethanol, 1 mL, by gavage, sacrificed 2 h later	10 mg/kg cysteine; 8.4 mg/kg NaHS; by gavage 2 h prior to ethanol	Gastric ulcers	N/A	Chávez-Piña et al. [97]
Wistar rats	5.25 g/kg ethanol in 27.8 mL/kg milk, by gavage, postnatal day 2–10	1 mg/kg NaHS concurrent with ethanol in milk	Hippocampal apoptosis, impaired spatial memory	BDNF, Brdu, Apoptosis	Mohseni et al. [98]
Wistar rats	5.25 g/kg ethanol in 27.8 mL/kg milk, by gavage, postnatal day 2–10	1 mg/kg NaHS concurrent with ethanol in milk	Reactive gliosis, necrosis and apoptosis of the hippocampus	AOE/ROS, TNF/Il, GFAP, Cas	Mohseni et al. [99]
Sprague–Dawley rats	56% (*v/v*) ethanol, 10 mL/kg daily for 8 weeks, gavage	25–100 mg/kg DATS, daily, gavage	Liver damage	AOE/ROS, Cas/Bcl2, CSE	Chen et al. [100]

4.2.2. H2S Improves Alcoholic Cardiomyopathy

Alcohol abuse is a major risk factor for the incidence of alcoholic cardiomyopathy and heart disorders. H2S is widely reported to provide a significant cardioprotection through its anti-apoptotic, anti-inflammatory, and antioxidant effects [21–23]. Accumulated evidence shows that H2S improved alcoholic cardiomyopathy. Long-term feeding of the mice with ethanol diet induced irregular arrangement of myocardial fibers and myocardial fibrosis, which could be significantly improved by the administration of H2S but deteriorated by PPG treatment. By targeting at miR-21 and miR-221-mediated TGF-β/PI3K/AKT signaling pathways, H2S inhibited autophagy-associated proteins (Beclin 1, Atg3, and Atg7) and fibrosis-associated proteins, thus relieving myocardial fibrosis in mice with alcoholic cardiomyopathy [89]. H2S has also been shown to protect against chronic alcohol intake-induced left ventricular remodeling in rats via attenuating oxidative stress and apoptosis [90].

4.2.3. H2S Attenuates Alcohol-Induced Gastric Ulcers

Gastric ulcer is the most common disease caused by alcohol consumption [91]. Accumulated evidence indicated that gastric ulcers by excessive consumption of alcohol can be prevented by many H2S donors or activators. Either NaHS or cysteine treatment dose-dependently prevented ethanol-induced macroscopic and microscopic gastric damage in mice by activating K_{ATP} channels and afferent neurons/TRPV1 receptors [92]. Consistent with these findings, Souza et al. also reported that pretreatment with d-cysteine increases the synthesis of H2S and protects from ethanol-induced oxidative stress and gastric lesions in mice [93]. AICAR as an AMPK activator prevented ethanol-induced gastric injury in mice by stimulating H2S generation [94]. Diligustilide, a dimeric phthalide isolated from *Ligusticum porteri*, is a traditional medicine for treating many diseases including gastric aches. Velázquez-Moyado et al. reported that diligustilide increased gastric H2S production and prevented ethanol-induced gastric injury [95]. Carbenoxolone, an anti-ulcer drug, protected from ethanol-induced lesions in rat stomachs via boosting endogenous H2S generation [96]. In contrast, Chávez-Piña et al. demonstrated that injection of a CSE inhibitor PPG suppressed H2S generation and reversed gastric injury caused by ethanol [97]. The

possible explanation may be due to the difference of animal types and doses of ethanol and H_2S used in each individual study.

4.2.4. H_2S Blocks Postnatal Alcohol Exposure-Induced Brain Damage

Maternal alcohol consumption during pregnancy often causes problems in fetal brain development and leads to fetal alcohol spectrum disorder. Mohseni et al. proved that supplementation of H_2S to rat pups with postnatal ethanol exposure protected from ethanol-induced neurotoxicity and memory loss, mostly due to the proneurogenesis and anti-apoptotic activity of H_2S [98]. The same group also reported that H_2S attenuated oxidative–inflammatory cascade and neuronal cell death by ethanol exposure in rat pups during the postnatal period [99]. This evidence suggests that H_2S is able to improve behavioral and cognitive deficits caused by postnatal alcohol exposure in rat pups. The protective role of H_2S against brain damage and dysfunction in adult animals with long-term consumption of alcohol need to be further studied.

4.3. Cellular Studies

Alcohol that is mostly metabolized in liver and alcohol consumption often causes liver damage. After incubation with ethanol, human hepatocyte cells exhibited increased apoptosis. It was further found that ethanol inhibited the protein expressions of CSE and CBS, and reduced endogenous H_2S generation in hepatocyte cells, while diallyl trisulfide boosted H_2S level and protected against ethanol-induced oxidative stress and apoptosis [100]. After depletion of intracellular cysteine by buthionine sulfoximine, ethanol induced more cell death in human hepatocyte cells, while exogenous glutathione supplementation led to increased cysteine level and cell viability [101]. In another study with rat liver cells, ethanol incubation activated the caspase 3-dependent apoptosis, and the presence of *S*-allyl-l-cysteine was sufficient to prevent cell death induced by ethanol [102]. Consumption of alcohol also leads to adipose tissue inflammation, hyperlipolysis, and apoptosis. Kema et al. reported that diallyl sulfide attenuated ethanol-induced oxidative stress, endoplasmic reticulum stress, and inflammation in 3T3L1 adipocyte cells, and promoted synthesis of M2 phenotype-specific genes in ethanol-exposed RAW 264.7 macrophage cells [103,104]. One study reported that ethanol exposure increased DNA damage of human bronchial epithelial cells, which could be attenuated by diallyl trisulfide pretreatment [105]. These results suggest that H_2S signaling may be a target for preventing and/or treating ethanol-induced injury of liver and adipose tissues, and possibly lung tissue.

5. Prospective

Increasing research has been performed in the discovery of new molecules and mechanisms for assisting in the diagnosis and treatment of diseases associated with cigarette smoking and alcohol abuse. H_2S is a novel gasotransmitter with great significance in human health. The disrupted H_2S signaling by cigarette smoking and alcohol drinking has recently come to light. The evidence from cellular and animal studies and also clinical observations identify H_2S as a regulator of oxidative stress and inflammatory response in the pathogenesis of various diseases associated with cigarette smoking and alcohol drinking. The mechanisms mediating the potential interactions among H_2S signaling, cigarette smoking, and alcohol abuse in different organs are still incompletely understood. A greater understanding of altered H_2S signaling can definitely help for developing novel diagnostic approaches. Moreover, the mechanisms underlying the altered expressions and activities of H_2S-generating enzymes have not been fully clarified. The notion of using fast H_2S-releasing donors to alter organ dysfunction should be treated with caution. Much work remains to be performed to develop slow H_2S-releasing donors. Furthermore, more clinical studies with a larger sample size and control subjects can help better characterize the disrupted H_2S signaling by cigarette smoking and alcohol drinking. There is also a need for accurate measurement of H_2S level and the activities of H_2S-generating enzymes in the affected organs by cigarette smoking and/or alcohol usage. Consequently, more

research needs to be undertaken to fully determine the potential of targeting H_2S signaling for prevention and treatment of cigarette smoking- and alcohol-associated diseases.

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References

1. Mokdad, A.H.; Marks, J.S.; Stroup, D.F.; Gerberding, J.L. Actual causes of death in the United States, 2000. *JAMA* **2004**, *291*, 1238–1245. [CrossRef] [PubMed]
2. Münzel, T.; Hahad, O.; Daiber, A. Double hazard of smoking and alcohol on vascular function in adolescents. *Eur. Heart J.* **2019**, *40*, 354–356. [CrossRef] [PubMed]
3. Manthey, J.; Imtiaz, S.; Neufeld, M.; Rylett, M.; Rehm, J. Quantifying the global contribution of alcohol consumption to cardiomyopathy. *Popul. Health Metr.* **2017**, *15*, 20. [CrossRef] [PubMed]
4. Wozniak, J.R.; Mueller, B.A.; Mattson, S.N.; Coles, C.D.; Kable, J.A.; Jones, K.L.; Boys, C.J.; Lim, K.O.; Riley, E.P.; Sowell, E.R.; et al. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). *Brain Imaging Behav.* **2017**, *11*, 1432–1445. [CrossRef]
5. O'Malley, S.S.; Zweben, A.; Fucito, L.M.; Wu, R.; Piepmeier, M.E.; Ockert, D.M.; Bold, K.W.; Petrakis, I.; Muvvala, S.; Jatlow, P.; et al. Effect of varenicline combined with medical management on alcohol use disorder with comorbid cigarette smoking: A randomized clinical trial. *JAMA Psychiatry* **2018**, *75*, 129–138. [CrossRef]
6. Valerio, G.; Mozzillo, E.; Zito, E.; De Nitto, E.; Maltoni, G.; Marigliano, M.; Zucchini, S.; Maffeis, C.; Franzese, A. Alcohol consumption or cigarette smoking and cardiovascular disease risk in youth with type 1 diabetes. *Acta Diabetol.* **2019**, *56*, 1315–1321. [CrossRef]
7. Miller, N.S.; Gold, M.S. Comorbid cigarette and alcohol addiction: Epidemiology and treatment. *J. Addict. Dis.* **1998**, *17*, 55–66. [CrossRef]
8. Gastfriend, D.R.; Garbutt, J.C.; Pettinati, H.M.; Forman, R.F. Reduction in heavy drinking as a treatment outcome in alcohol dependence. *J. Subst. Abuse Treat.* **2007**, *33*, 71–80. [CrossRef]
9. Asay, G.R.B.; Homa, D.M.; Abramsohn, E.M.; Xu, X.; O'Connor, E.L.; Wang, G. Reducing smoking in the US federal workforce: 5-year health and economic impacts from improved cardiovascular disease outcomes. *Public Health Rep.* **2017**, *132*, 646–653. [CrossRef]
10. Lieber, C.S. Alcoholic fatty liver: Its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol* **2004**, *34*, 9–19. [CrossRef]
11. Lee, J.; Taneja, V.; Vassallo, R. Cigarette smoking and inflammation: Cellular and molecular mechanisms. *J. Dent. Res.* **2012**, *91*, 142–149. [CrossRef] [PubMed]
12. Aseervatham, S.; Choi, S.; Krishnan, J.; Ruckmani, K. Cigarette smoke and related risk factors in neurological disorders: An update. *Biomed. Pharmacother.* **2017**, *85*, 79–86.
13. Paquot, N. The metabolism of alcohol. *Rev. Med. Liege* **2019**, *74*, 265–267. [PubMed]
14. Song, I.H.; Jung, K.J.; Lee, T.J.; Kim, J.Y.; Sung, E.G.; Bae, Y.C.; Park, Y.H. Mesenchymal stem cells attenuate adriamycin-induced nephropathy by diminishing oxidative stress and inflammation via downregulation of the NF-kB. *Nephrology* **2018**, *23*, 483–492. [CrossRef]
15. Li, X.; Kovacs, E.J.; Schwacha, M.G.; Chaudry, I.H.; Choudhry, M.A. Acute alcohol intoxication increases interleukin-18-mediated neutrophil infiltration and lung inflammation following burn injury in rats. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2007**, *292*, L1193–L1201. [CrossRef]
16. Doz, E.; Noulin, N.; Boichot, E.; Guénon, I.; Fick, L.; Bert, M.L.; Lagente, V.; Ryffel, B.; Schnyder, B.; Quesniaux, V.F.; et al. Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. *J. Immun.* **2008**, *180*, 1169–1178. [CrossRef]
17. Dhami, R.; Gilks, B.; Xie, C.; Zay, K.; Wright, J.L.; Churg, A. Acute cigarette smoke–induced connective tissue breakdown is mediated by neutrophils and prevented by α 1-Antitrypsin. *Am. J. Respir. Cell. Mol. Biol.* **2000**, *22*, 244–252. [CrossRef]
18. Ramaiah, S.K.; Jaeschke, H. Hepatic neutrophil infiltration in the pathogenesis of alcohol-induced liver injury. *Toxicol. Mech. Methods* **2007**, *17*, 431–440. [CrossRef]

19. Cooper, C.E.; Brown, G.C. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: Chemical mechanism and physiological significance. *J. Bioenerg. Biomembr.* **2008**, *40*, 533–539. [CrossRef]

20. Abe, K.; Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* **1996**, *16*, 1066–1071. [CrossRef]

21. Wang, R. The gasotransmitter role of hydrogen sulfide. *Antioxid. Redox Signal.* **2003**, *5*, 493–501. [CrossRef] [PubMed]

22. Wang, R. Two's company, three's a crowd: Can H$_2$S be the third endogenous gaseous transmitter? *FASEB J.* **2002**, *16*, 1792–1798. [CrossRef] [PubMed]

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

24. Sen, U.; Sathnur, P.B.; Kundu, S.; Givvimani, S.; Coley, D.M.; Mishra, P.K.; Qipshidze, N.; Tyagi, N.; Metreveli, N.; Tyagi, S.C. Increased endogenous H$_2$S generation by CBS, CSE, and 3MST gene therapy improves ex vivo renovascular relaxation in hyperhomocysteinemia. *Am. J. Physiol. Cell. Physiol.* **2012**, *303*, 41–51. [CrossRef] [PubMed]

25. Mustafa, A.K.; Gadalla, M.M.; Sen, N.; Kim, S.; Mu, W.; Gazi, S.K.; Barrow, R.K.; Yang, G.; Wang, R.; Snyder, S.H. H$_2$S signals through protein S-sulfhydration. *Sci. Signal.* **2009**, *96*, ra72. [CrossRef] [PubMed]

26. Zhao, K.; Li, H.; Li, S.; Yang, G. Regulation of cystathionine gamma-lyase/H$_2$S system and its pathological implication. *Front. Biosci.* **2014**, *19*, 1355–1369. [CrossRef] [PubMed]

27. Yang, G.; Zhao, K.; Ju, Y.; Mani, S.; Cao, Q.; Puukila, S.; Khaper, N.; Wu, L.; Wang, R. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. *Antioxid. Redox Signal.* **2012**, *18*, 1906–1919. [CrossRef]

28. Ju, Y.; Fu, M.; Stokes, E.; Wu, L.; Yang, G. H$_2$S-mediated protein S-sulfhydration: A prediction for its formation and regulation. *Molecules* **2017**, *22*, 1334. [CrossRef]

29. Paul, B.D.; Snyder, S.H. H$_2$S: A novel gasotransmitter that signals by sulfhydration. *Trends Biochem. Sci.* **2015**, *40*, 687–700. [CrossRef]

30. Koike, S.; Ogasawara, Y.; Shibuya, N.; Kimura, H.; Ishii, K. Polysulfide exerts a protective effect against cytotoxicity caused by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells. *FEBS Lett.* **2013**, *587*, 3548–3555. [CrossRef]

31. Hourihan, J.M.; Kenna, J.G.; Hayes, J.D. The gasotransmitter hydrogen sulfide induces Nrf2-target genes by inactivating the Keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between Cys-226 and Cys-613. *Antioxid. Redox Signal.* **2012**, *19*, 465–481. [CrossRef] [PubMed]

32. Aroca, Á.; Serna, A.; Gotor, C.; Romero, L.C. S-sulfhydration: A cysteine posttranslational modification in plant systems. *Plant. Physiol.* **2015**, *168*, 334–342. [CrossRef] [PubMed]

33. Fu, L.; Liu, K.; He, J.; Tian, C.; Yu, X.; Yang, J. Direct proteomic mapping of cysteine persulfidation. *Antioxid. Redox Signal.* **2020**, *33*, 1061–1076. [CrossRef] [PubMed]

34. Iciek, M.; Bilska-Wilkosz, A.; Górny, M. Sulfane sulfur—New findings on an old topic. *Acta Biochim. Pol.* **2019**, *66*, 533–544. [CrossRef] [PubMed]

35. Akaike, T.; Ida, T.; Wei, F.; Nishida, M.; Kumagai, Y.; Alam, M.; Ihara, H.; Sawa, T.; Matsunaga, T.; Kasamatsu, S.; et al. Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat. Commun.* **2017**, *8*, 1177. [CrossRef] [PubMed]

36. Ju, Y.; Wu, L.; Yang, G. Thioredoxin 1 regulation of protein S-desulfhydration. *Biochem. Biophys. Rep.* **2015**, *5*, 27–34. [CrossRef] [PubMed]

37. Motohashi, H.; Yamamoto, M. Nrf2–Keap1 defines a physiologically important stress response mechanism. *Trends Mol. Med.* **2004**, *10*, 549–557. [CrossRef]

38. Itoh, K.; Chiba, T.; Takahashi, S.; Ishii, T.; Igarashi, K.; Katoh, Y.; Oyake, T.; Hayashi, N.; Satoh, K.; Hatayama, I.; et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* **1997**, *236*, 313–322. [CrossRef]

39. Kimura, Y.; Goto, Y.I.; Kimura, H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid. Redox Signal.* **2009**, *12*, 1–13. [CrossRef]

40. Ida, T.; Sawa, T.; Ihara, H.; Tsuchiya, Y.; Watanabe, Y.; Kumagai, Y.; Suematsu, M.; Motohashi, H.; Fujii, S.; Matsunaga, T.; et al. Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7606–7611. [CrossRef]

41. Liu, X.; Liu, X.; Jiang, P.; Huang, H.; Yan, Y. Plasma levels of endogenous hydrogen sulfide and homocysteine in patients with Alzheimer's disease and vascular dementia and the significance thereof. *Zhonghua Yi Xue Za Zhi.* **2008**, *88*, 2246–2249. [PubMed]

42. 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] [PubMed]

43. 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*, 667–674. [CrossRef] [PubMed]

44. Ali, A.; Zhang, Y.; Fu, M.; Pei, Y.; Wu, L.; Wang, R.; Yang, G. Cystathionine gamma-lyase/H$_2$S system suppresses hepatic acetyl-CoA accumulation and nonalcoholic fatty liver disease in mice. *Life Sci.* **2020**, *252*, 117661. [CrossRef]

45. Chen, Y.H.; Yao, W.Z.; Geng, B.; Ding, Y.L.; Lu, M.; Zhao, M.W.; Tang, C.S. Endogenous hydrogen sulfide in patients with COPD. *Chest* **2005**, *128*, 3205–3211. [CrossRef]

46. Sun, Y.; Wang, K.; Li, M.X.; He, W.; Chang, J.R.; Liao, C.C.; Lin, F.; Qi, Y.F.; Wang, R.; Chen, Y.H. Metabolic changes of H₂S in smokers and patients of COPD which might involve in inflammation, oxidative stress and steroid sensitivity. *Sci. Rep.* **2015**, *5*, 14971. [CrossRef]

47. Saito, J.; Mackay, A.J.; Rossios, C.; Gibeon, D.; Macedo, P.; Sinharay, R.; Bhavsar, P.K.; Wedzicha, J.A.; Chung, K.F. Sputum-to-serum hydrogen sulfide ratio in COPD. *Thorax* **2014**, *69*, 903–909. [CrossRef]

48. Sun, Y.; Wang, X.M.; Chen, Y.H.; Zhu, R.X.; Liao, C.C. Exhaled hydrogen sulfide in patients with chronic obstructive pulmonary disease and its correlation with exhaled nitric oxide. *Chin. Med. J.* **2013**, *126*, 3240–3244.

49. Zhang, J.; Wang, X.; Chen, Y.; Yao, W. Exhaled Hydrogen Sulfide Predicts Airway Inflammation Phenotype in COPD. *Respir. Care* **2015**, *60*, 251–258. [CrossRef]

50. Carubbi, C.; Masselli, E.; Calabrò, E.; Bonati, E.; Galeone, C.; Andreoli, R.; Goldoni, M.; Corradi, M.; Sverzellati, N.; Pozzi, G.; et al. Sulphurous thermal water inhalation impacts respiratory metabolic parameters in heavy smokers. *Int. J. Biometeorol.* **2019**, *63*, 1209–1216. [CrossRef]

51. Bates, M.N.; Crane, J.; Balmes, J.R.; Garrett, N. Investigation of hydrogen sulfide exposure and lung function, asthma and chronic obstructive pulmonary disease in a geothermal area of New Zealand. *PLoS ONE* **2015**, *10*, e0122062. [CrossRef] [PubMed]

52. Singh, S.P.; Devadoss, D.; Manevski, M.; Sheybani, A.; Ivanciuc, T.; Exil, V.; Agarwal, H.; Raizada, V.; Garofalo, R.P.; Chand, H.S.; et al. Gestational exposure to cigarette smoke suppresses the gasotransmitter H(2)S biogenesis and the effects are transmitted transgenerationally. *Front. Immunol.* **2020**, *11*, 1628. [CrossRef] [PubMed]

53. Zhang, S.; Lei, F.; Zhao, F.; Yan, X.; Wang, W.; Zheng, Y. Hydrogen sulfide ameliorates prenatal cigarette smoke exposure-induced impairment of respiratory responses to hypercapnia in neonatal rats. *Respir. Physiol. Neurobiol.* **2017**, *243*, 1–6. [CrossRef] [PubMed]

54. Lei, F.; Wang, W.; Fu, Y.; Wang, J.; Zheng, Y. Mitochondrial KATP channels contribute to the protective effects of hydrogen sulfide against impairment of central chemoreception of rat offspring exposed to maternal cigarette smoke. *PLoS ONE* **2020**, *15*, e0237643. [CrossRef]

55. Nie, L.H.; Hu, Y.J.; Li, X.K.; Xue, L.; Jia, Q.Y.; Yang, Y.W.; Zheng, Y. Effects of intrauterine cigarette smoking exposure on expression of 3-mercaptopyruvate sulfurtransferase in medulla oblongata of neonatal rats. *Sichuan Da Xue Xue Bao Yi Xue Ban* **2013**, *44*, 526–530.

56. Wang, J.; Lei, F.; Fu, Y.T.; Zheng, Y. Effect of prenatal cigarette smoke exposure on sevoflurane-induced respiratory suppression in neonatal rats and the protective role of hydrogen sulfide. *Respir. Physiol. Neurobiol.* **2020**, *13*, 103582.

57. Yan, X.; Zhao, F.; Zhang, S.; Lei, F.; Wang, W.; Zheng, Y. Hydrogen sulfide ameliorates disorders in the parafacial respiratory group region of neonatal rats caused by prenatal cigarette smoke exposure via an antioxidative effect. *Environ. Toxicol. Pharmacol.* **2019**, *68*, 80–90. [CrossRef]

58. Yan, X.; Lei, F.; Hu, Y.; Nie, L.; Jia, Q.; Zhou, H.; Zhao, F.; Zheng, Y. Hydrogen sulfide protects neonatal rat medulla oblongata against prenatal cigarette smoke exposure via anti-oxidative and anti-inflammatory effects. *Environ. Toxicol. Pharmacol.* **2018**, *57*, 151–158. [CrossRef]

59. Zhao, F.; Lei, F.; Yan, X.; Zhang, S.; Wang, W.; Zheng, Y. Protective effects of hydrogen sulfide against cigarette smoke exposure-induced placental oxidative damage by alleviating redox imbalance via Nrf2 pathway in rats. *Cell Physiol. Biochem.* **2018**, *48*, 1815–1828. [CrossRef]

60. Zhao, F.; Lei, F.; Zhang, S.; Yan, X.; Wang, W.; Zheng, Y. Hydrogen sulfide alleviates placental injury induced by maternal cigarette smoke exposure during pregnancy in rats. *Nitric Oxide* **2018**, *74*, 102–111. [CrossRef]

61. Guan, R.; Wang, J.; Cai, Z.; Li, Z.; Wang, L.; Li, Y.; Xu, J.; Li, D.; Yao, H.; Liu, W.; et al. Hydrogen sulfide attenuates cigarette smoke-induced airway remodeling by upregulating SIRT1 signaling pathway. *Redox Biol.* **2020**, *28*, 101356. [CrossRef] [PubMed]

62. Han, W.; Dong, Z.; Dimitropoulou, C.; Su, Y. Hydrogen Sulfide Ameliorates Tobacco Smoke-Induced Oxidative Stress and Emphysema in Mice. *Antioxid. Redox Signal.* **2011**, *15*, 2121–2134. [CrossRef] [PubMed]

63. Wang, L.; Meng, J.; Wang, C.; Yang, C.; Wang, Y.; Li, Y.; Li, Y. Hydrogen sulfide alleviates cigarette smoke-induced COPD through inhibition of the TGF-beta1/smad pathway. *Exp. Biol. Med.* **2020**, *245*, 190–200. [CrossRef] [PubMed]

64. Ivanciuc, T.; Sbrana, E.; Casola, A.; Garofalo, R.P. Cystathionine γ-lyase deficiency enhances airway reactivity and viral-induced disease in mice exposed to side-stream tobacco smoke. *Pediatr. Res.* **2019**, *86*, 39–46. [CrossRef] [PubMed]

65. Li, M.X.; Chen, Y.H.; Liao, C.C.; Lin, F.; Bai, Y.; Mi, W.J.; Sun, Y.; Qi, Y.F. Role and mechanism of hydrogen sulfide in cigarette smoke induced chronic obstructive pulmonary disease related pulmonary vascular remodeling in rats. *Zhonghua Yi Xue Za Zhi* **2017**, *97*, 137–142. [PubMed]

66. Hartmann, C.; Hafner, S.; Scheuerle, A.; Möller, P.; Huber-Lang, M.; Jung, B.; Nubaum, B.; McCook, O.; Gröger, M.; Wagner, F.; et al. The role of cystathionine-gamma-lyase in blunt chest trauma in cigarette smoke exposed mice. *Shock* **2017**, *47*, 491–499. [CrossRef]

67. Davis, J.A.; Brown, A.T.; Chen, H.; Wang, Y.; Poirier, L.A.; Eidt, J.F.; Cruz, C.P.; Moursi, M.M. Cigarette smoke increases intimal hyperplasia and homocysteine in a rat carotid endarterectomy. *J. Surg. Res.* **2004**, *121*, 69–75. [CrossRef]

68. Chen, Y.H.; Wang, P.P.; Wang, X.M.; He, Y.J.; Yao, W.Z.; Qi, Y.F.; Tang, C.S. Involvement of endogenous hydrogen sulfide in cigarette smoke-induced changes in airway responsiveness and inflammation of rat lung. *Cytokine* **2011**, *53*, 334–341. [CrossRef]

69. Zhou, X.; Zhao, L.; Mao, J.; Huang, J.; Chen, J. Antioxidant effects of hydrogen sulfide on left ventricular remodeling in smoking rats are mediated via PI3K/Akt-dependent activation of Nrf2. *Toxicol. Sci.* **2015**, *144*, 197–203. [CrossRef]

70. Zhou, X.; An, G.; Chen, J. Hydrogen sulfide improves left ventricular function in smoking rats via regulation of apoptosis and autophagy. *Apoptosis* **2014**, *19*, 998–1005. [CrossRef]

71. Wiliński, J.; Wiliński, B.; Somogyi, E.; Piotrowska, J.; Kameczura, T.; Zygmunt, M. Nicotine affects hydrogen sulfide concentrations in mouse kidney and heart but not in brain and liver tissues. *Folia Med.* **2017**, *57*, 55–64.

72. Li, H.; Teng, X.; Yang, R.; Guo, Q.; Xue, H.; Xiao, L.; Duan, X.; Tian, D.; Feng, X.; Wu, Y. Hydrogen sulfide facilitates the impaired sensitivity of carotid sinus baroreflex in rats with vascular calcification. *Front. Pharmacol.* **2017**, *8*, 629. [CrossRef] [PubMed]

73. Zhang, H.T.; Zhang, T.; Chai, M.; Sun, J.J.; Yu, X.Y.; Liu, C.Z.; Huang, C.C. Effect of tobacco smoke on hydrogen sulfide-induced rat thoracic aorta relaxation. *Braz. J. Med. Biol. Res.* **2017**, *50*, e5592. [CrossRef] [PubMed]

74. Guan, R.; Cai, Z.; Wang, J.; Ding, M.; Li, Z.; Xu, J.; Li, Y.; Li, J.; Yao, H.; Liu, W.; et al. Hydrogen sulfide attenuates mitochondrial dysfunction-induced cellular senescence and apoptosis in alveolar epithelial cells by upregulating sirtuin 1. *Aging* **2019**, *11*, 11844–11864. [CrossRef] [PubMed]

75. Guan, R.; Wang, J.; Li, D.; Li, Z.; Liu, H.; Ding, M.; Cai, Z.; Liang, X.; Yang, Q.; Long, Z.; et al. Hydrogen sulfide inhibits cigarette smoke-induced inflammation and injury in alveolar epithelial cells by suppressing PHD2/HIF-1α/MAPK signaling pathway. *Int. Immunopharmacol.* **2020**, *81*, 105979. [CrossRef]

76. Lin, F.; Liao, C.; Sun, Y.; Zhang, J.; Lu, W.; Bai, Y.; Liao, Y.; Li, M.; Ni, X.; Hou, Y.; et al. Hydrogen sulfide inhibits cigarette smoke-induced endoplasmic reticulum stress and apoptosis in bronchial epithelial cells. *Front. Pharmacol.* **2017**, *8*, 675. [CrossRef]

77. Perry, M.M.; Tildy, B.; Papi, A.; Casolari, P.; Caramori, G.; Rempel, K.L.; Halayko, A.J.; Adcock, I.; Chung, K.F. The anti-proliferative and anti-inflammatory response of COPD airway smooth muscle cells to hydrogen sulfide. *Respir. Res.* **2018**, *19*, 85. [CrossRef]

78. Lee, S.K.; Chung, J.H.; Choi, S.C.; Auh, Q.S.; Lee, Y.M.; Lee, S.I.; Kim, E.C. Sodium hydrogen sulfide inhibits nicotine and lipopolysaccharide-induced osteoclastic differentiation and reversed osteoblastic differentiation in human periodontal ligament cells. *J. Cell Biochem.* **2013**, *114*, 1183–1193. [CrossRef]

79. Mizrak, S.; Turan, V.; Inan, S.; Uysal, A.; Yilmaz, C.; Ercan, G. Effect of nicotine on RANKL and OPG and bone mineral density. *J. Invest. Surg.* **2014**, *27*, 327–331. [CrossRef]

80. Suzuki, N.; Yoneda, M.; Naito, T.; Iwamoto, T.; Yamada, K.; Hisama, K.; Okada, I.; Hirofuji, T. The relationship between alcohol consumption and oral malodour. *Int. Dent. J.* **2009**, *59*, 31–34.

81. Matsumoto, H.; Matsubayashi, K.; Fukui, Y. Evidence that cytochrome P-4502E1 contributes to ethanol elimination at low doses: Effects of diallyl sulfide and 4-methyl pyrazole on ethanol elimination in the perfused rat liver. *Alcohol Clin. Exp. Res.* **1996**, *20*, 12A–16A. [CrossRef] [PubMed]

82. Zeng, T.; Zhang, C.L.; Zhu, Z.P.; Yu, L.H.; Zhao, X.L.; Xie, K.Q. Diallyltrisulfide (DATS) effectively attenuated oxidative stress-mediated liver injury and hepatic mitochondrial dysfunction in acute ethanol-exposed mice. *Toxicology* **2008**, *252*, 86–91. [CrossRef] [PubMed]

83. Chen, L.Y.; Chen, Q.; Cheng, Y.F.; Jin, H.H.; Kong, D.S.; Zhang, F.; Wu, L.; Shao, J.; Zheng, S.Z. Diallyltrisulfide attenuates ethanol-induced hepatic steatosis by inhibiting oxidative stress and apoptosis. *Biomed. Pharmacother.* **2016**, *79*, 35–43. [CrossRef] [PubMed]

84. Zeng, T.; Zhang, C.L.; Song, F.Y.; Zhao, X.L.; Yu, L.H.; Zhu, Z.P.; Xie, K.Q. The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress. *Biochim. Biophys. Acta* **2013**, *1830*, 4848–4859. [CrossRef] [PubMed]

85. Zeng, T.; Zhang, C.L.; Song, F.Y.; Zhao, X.L.; Xie, K.Q. Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR-α, and CYP2E1. *Food Chem. Toxicol.* **2012**, *50*, 485–491. [CrossRef]

86. Wang, Y.; Guan, M.; Zhao, X.; Li, X. Effects of garlic polysaccharide on alcoholic liver fibrosis and intestinal microflora in mice. *Pharm. Biol.* **2018**, *56*, 325–332. [CrossRef]

87. Ronis, M.J.; Korourian, S.; Blackburn, M.L.; Badeaux, J.; Badger, T.M. The role of ethanol metabolism in development of alcoholic steatohepatitis in the rat. *Alcohol* **2010**, *44*, 157–169. [CrossRef]

88. Iciek, M.; Górny, M.; Bilska-Wilkosz, A.; Kowalczyk-Pachel, D. Is aldehyde dehydrogenase inhibited by sulfur compounds? In vitro and in vivo studies. *Acta Biochim. Pol.* **2018**, *65*, 125–132. [CrossRef]

89. Liang, B.; Xiao, T.; Long, J.; Liu, M.; Li, Z.; Liu, S.; Yang, J. Hydrogen sulfide alleviates myocardial fibrosis in mice with alcoholic cardiomyopathy by downregulating autophagy. *Int. J. Mol. Med.* **2017**, *40*, 1781–1791. [CrossRef]

90. Zhou, X.; Lu, X.; Xu, W.; Chen, J. Protective effects of hydrogen sulfide against chronic alcohol intake-induced left ventricular remodeling in rats. *Cardiovasc. Drugs Ther.* **2013**, *27*, 221–227. [CrossRef]

91. Rahman, Z.; Dwivedi, D.K.; Jena, G.B. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. *Hum. Exp. Toxicol.* **2020**, *39*, 547–562. [CrossRef]

92. Medeiros, J.V.; Bezerra, V.H.; Gomes, A.S.; Barbosa, A.L.; Lima-Júnior, R.C.; Soares, P.M.; Brito, G.A.; Ribeiro, R.A.; Cunha, F.Q.; Souza, M.H. Hydrogen sulfide prevents ethanol-induced gastric damage in mice: Role of ATP-sensitive potassium channels and capsaicin-sensitive primary afferent neurons. *J. Pharm. Exp.* **2009**, *330*, 764–770. [CrossRef] [PubMed]

93. Souza, L.K.; Araújo, T.S.; Sousa, N.A.; Sousa, F.B.; Nogueira, K.M.; Nicolau, L.A.; Medeiros, J.V. Evidence that d-cysteine protects mice from gastric damage via hydrogen sulfide produced by d-amino acid oxidase. *Nitric Oxide* **2017**, *64*, 1–6. [CrossRef]

94. de Araújo, S.; Oliveira, A.P.; Sousa, F.B.M.; Souza, L.K.M.; Pacheco, G.; Filgueiras, M.C.; Nicolau, L.A.D.; Brito, G.A.C.; Cerqueira, G.S.; Silva, R.O.; et al. AMPK activation promotes gastroprotection through mutual interaction with the gaseous mediators H(2)S, NO, and CO. *Nitric Oxide* **2018**, *78*, 60–71. [CrossRef] [PubMed]

95. Velázquez-Moyado, J.A.; Balderas-López, J.L.; Pineda-Peña, E.A.; Sánchez-Ortiz, B.L.; Tavares-Carvalho, J.C.; Navarrete, A. Diligustilide releases H(2)S and stabilizes S-nitrosothiols in ethanol-induced lesions on rat gastric mucosa. *Inflammopharmacology* **2018**, *26*, 611–619. [CrossRef] [PubMed]

96. Velázquez-Moyado, J.A.; Navarrete, A. The detection and quantification, in vivo and in real time, of hydrogen sulfide in ethanol-induced lesions in rat stomachs using an ion sensitive electrode. *J. Pharmacol. Toxicol. Methods* **2018**, *89*, 54–58. [CrossRef] [PubMed]

97. Chávez-Piña, A.E.; Tapia-Alvarez, G.R.; Navarrete, A. Inhibition of endogenous hydrogen sulfide synthesis by PAG protects against ethanol-induced gastric damage in the rat. *Eur. J. Pharmacol.* **2010**, *630*, 131–136. [CrossRef]

98. Mohseni, F.; Bagheri, F.; Rafaiee, R.; Norozi, P.; Khaksari, M. Hydrogen sulfide improves spatial memory impairment via increases of BDNF expression and hippocampal neurogenesis following early postnatal alcohol exposure. *Physiol. Behav.* **2020**, *215*, 112784. [CrossRef]

99. Mohseni, F.; Bagheri, F.; Khaksari, M. Hydrogen sulfide attenuates the neurotoxicity in the animal model of fetal alcohol spectrum disorders. *Neurotox. Res.* **2020**, *37*, 977–986. [CrossRef]

100. Chen, L.Y.; Chen, Q.; Zhu, X.J.; Kong, D.S.; Wu, L.; Shao, J.J.; Zheng, S.Z. Diallyl trisulfide protects against ethanol-induced oxidative stress and apoptosis via a hydrogen sulfide-mediated mechanism. *Int. Immunopharmacol.* **2016**, *36*, 23–30. [CrossRef]

101. Kumar, S.M.; Haridoss, M.; Swaminathan, K.; Gopal, R.K.; Clemens, D.; Dey, A. The effects of changes in glutathione levels through exogenous agents on intracellular cysteine content and protein adduct formation in chronic alcohol-treated VL17A cells. *Toxicol. Mech. Methods* **2017**, *27*, 128–135. [CrossRef] [PubMed]

102. Chen, P.; Hu, M.; Liu, F.; Yu, H.; Chen, C. S-allyl-l-cysteine (SAC) protects hepatocytes from alcohol-induced apoptosis. *FEBS Open Bio* **2019**, *9*, 1327–1336. [CrossRef] [PubMed]

103. Kema, V.H.; Khan, I.; Kapur, S.; Mandal, P. Evaluating the effect of diallyl sulfide on regulation of inflammatory mRNA expression in 3T3L1 adipocytes and RAW 264.7 macrophages during ethanol treatment. *Drug Chem. Toxicol.* **2018**, *41*, 302–313. [CrossRef] [PubMed]

104. Kema, V.H.; Khan, I.; Jamal, R.; Vishwakarma, S.K.; Lakki Reddy, C.; Parwani, K.; Patel, F.; Patel, D.; Khan, A.A.; Mandal, P. Protective effects of diallyl sulfide against ethanol-induced injury in rat adipose tissue and primary human adipocytes. *Alcohol Clin. Exp. Res.* **2017**, *41*, 1078–1092. [CrossRef]

105. Sapkota, M.; Hottor, T.K.; DeVasure, J.M.; Wyatt, T.A.; McCaskill, M.L. Protective role of CYP2E1 inhibitor diallyl disulfide (DADS) on alcohol-induced malondialdehyde-deoxyguanosine (M1dG) adduct formation *Alcohol Clin*. *Exp. Res.* **2014**, *38*, 1550–1558. [CrossRef]