N-acetylcysteine (NAC) and Hydrogen Sulfide (H2S) in Coronavirus Disease 2019 (COVID-19)

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N-Acetylcysteine and Hydrogen Sulfide in Coronavirus Disease 2019

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Abstract

Significance: Hydrogen sulfide (H_2S) is one of the three main gasotransmitters that are endogenously produced in humans and are protective against oxidative stress. Recent findings from studies focusing on coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), shifted our attention to a potentially modulatory role of H_2S in this viral respiratory disease.
Recent Advances: H_2S levels at hospital admission may be of importance since this gasotransmitter has been shown to be protective against lung damage through its antiviral, antioxidant, and anti-inflammatory actions. Furthermore, many COVID-19 cases have been described demonstrating remarkable clinical improvement upon administration of high doses of N-acetylcysteine (NAC). NAC is a renowned pharmacological antioxidant substance acting as a source of cysteine, thereby promoting endogenous glutathione (GSH) biosynthesis as well as generation of sulfane sulfur species when desulfurated to H_2S.
Critical Issues: Combining H_2S physiology and currently available knowledge of COVID-19, H_2S is hypothesized to target three main vulnerabilities of SARS-CoV-2: (i) cell entry through interfering with functional host receptors, (ii) viral replication through acting on RNA-dependent RNA polymerase (RdRp), and (iii) the escalation of inflammation to a potentially lethal hyperinflammatory cytokine storm (toll-like receptor 4 [TLR4] pathway and NLR family pyrin domain containing 3 [NLRP3] inflammasome).
Future Directions: Dissecting the breakdown of NAC reveals the possibility of increasing endogenous H_2S levels, which may provide a convenient rationale for the application of H_2S-targeted therapeutics. Further randomized-controlled trials are warranted to investigate its definitive role. *Antioxid. Redox Signal.* 00, 000–000.

Keywords: hydrogen sulfide, COVID-19, N-acetylcysteine, taurine, reactive sulfur species

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has developed since December 2019 and quickly spread globally resulting in a pandemic with already over 1 million fatalities (61). In contrast to previous coronaviruses, SARS-CoV-2 is characterized by a relatively high infectivity, sometimes causing severe inflammatory disease (105). Clinical symptomatology of COVID-19 varies from mild, self-limiting disease with mainly respiratory complaints, to the development of pneumonia, respiratory

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failure with acute respiratory distress syndrome (ARDS), and even multiorgan failure (MOF) or death (104). Laboratory examination often reveals elevated levels of C-reactive protein (CRP), elevated liver transaminases, D-dimers, ferritin, lactate dehydrogenase (LDH), and lymphopenia. Some patients are more prone to develop severe disease requiring hospitalization, including older patients, males, and those with relevant comorbidity (*e.g.*, cardiovascular diseases, obesity, diabetes mellitus, chronic respiratory disease, and immune-mediated inflammatory diseases [IMIDs]) (11).

To date, no effective therapeutics are available to combat the disease. Current treatment strategies include supportive care with mechanical ventilation and oxygen supplementation, as well as the use of repurposed or symptomatic drugs, such as dexamethasone and anticoagulants. For instance, dexamethasone has recently anchored in the treatment on evidence-based grounds (42). Yet, no single antiviral or immune-supportive agent has proven efficacy upon clinical trial results. Likewise, vaccine development is still ongoing, but is accompanied by a risk of incomplete viral coverage due to the increasing number of identified SARS-CoV-2 mutants, compromised immunogenicity (especially in older, adipose, and immunodeficient people), and failure of a durable immune response. Considering all these issues, it is of utmost importance to encourage performance of well-designed randomized-controlled trials (RCTs) to establish effective and disease stage-specific therapy.

Recently, some studies provided evidence for a potentially modulatory role of hydrogen sulfide (H_2S) in COVID-19 (17, 21, 28, 83, 108). Intravenous administration of the antioxidant, cysteine prodrug and H_2S donor N-acetylcysteine (NAC) led to remarkable clinical and biochemical improvement in 10 consecutive patients with severe COVID-19 (45). This potential therapeutic effect of NAC has been repeatedly demonstrated in several other reports, and clinical trials are currently on their way to further examine the utility of this drug (43, 81).

In this review, we aim to outline a rationale for potential therapeutic application of H_2S(-donor) supplementation in COVID-19. To do so, it is crucial to carefully dissect the mechanisms of NAC and other possible H_2S-promoting substances such as taurine or taurolidine to gain insight into their contribution in the modulation of endogenous H_2S levels. In this review, we describe H_2S as an antiviral host factor, discuss possibilities to enhance H_2S levels, and highlight the hypothesized multifaceted antiviral action of H_2S against SARS-CoV-2.

Physiology of H_2S

Endogenous production of H_2S

H_2S is endogenously produced out of sulfur-containing amino acids (SAAs) such as cysteine. Four major synthetic pathways exist that result in the production of H_2S in mammals. Two cytosolic enzymes, cystathionine-β-synthase (CBS) and cystathionine gamma-lyase (CSE), functioning within the transsulfuration pathway, are pyridoxal-5′-phosphate (PLP, the active form of vitamin B_6)-dependent enzymes and crucial for H_2S synthesis. CBS and CSE are widely expressed throughout the human body, among others in the liver, kidney, brain, ileum, and vascular tissue. CBS is predominantly active within the nervous system, whereas CSE is mainly observed in vascular smooth muscle cells and the heart (116). Another H_2S-synthesizing pathway exists primarily in mitochondria, and is a cysteine catabolic pathway mediated by cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST). Finally, production of H_2S can also occur nonenzymatically through reductive chemistry of various sulfur species, including thiosulfate, thiocystine, and sulfite, although these mechanisms are less well understood and account for only a small part of H_2S production (1). Recently, however, another nonenzymatic H_2S-generating mechanism has been described, which involves a nonenzymatic catalysis of cysteine by coordinated action of iron (Fe^{3+} form) and PLP (110). H_2S production in the human body is thought to be regulated by the hypothalamic/pituitary axis by negative regulation of growth hormone (GH) and thyroid hormone (TH) signaling as has been observed in mice (41). An overview of endogenous H_2S synthesis pathways is given in Figure 1.

(Patho)-physiological functions of H_2S

H_2S belongs to a family of labile biological mediators. They share many similarities such as the rapid transfer through cell membranes without the use of specific transporters. H_2S is recognized as a crucial signaling molecule that exerts a variety of (patho)-physiological effects. First, H_2S is a physiological vasorelaxant by activation of adenosine triphosphate-sensitive potassium (K^{ATP}) channels through cysteine S-sulfhydration in vascular endothelial cells and vascular smooth muscle cells (72, 109). H_2S is believed to have a biphasic effect on the vascular tone, with lower concentrations of H_2S inducing vasoconstriction. This postulation is supported by the fact that reversal of the vasodilatory effect of acetylcholine and histamine, which are both nitric oxide (NO)-dependent vasodilators, was achieved after treatment with H_2S in lower concentrations ($<100\ \mu M$) (5). Furthermore, H_2S protects against oxidative stress, both as a direct scavenger of oxidants and by upregulating the antioxidant defense system, thus preventing cytokine- or oxidant-induced oxidative damage in various tissues (95). H_2S is also known to upregulate heme oxygenase-1 (HO-1), glutathione (GSH), and superoxide dismutase 1 and 2 (SOD1 and SOD2), and downregulate toll-like receptor 4/nuclear factor kappa-light-chain-enhancer of activated B cell (TLR4/NF-κB) activation, thereby inhibiting proinflammatory factors (73, 77, 115). For the remaining, H_2S has cytoprotective, antiangiogenic, antiapoptotic, and antifibrotic effects (14, 85). A lack of H_2S is known to be involved in cardiovascular diseases, neurodegenerative diseases, and cancer. The most extensively studied functions of endogenously produced H_2S relate to its vasodilatory effects and to its ability to lower and modulate oxidative stress. Several other physiological functions of H_2S signaling have been proposed. Most of these studies showed that H_2S has the potential to exhibit beneficial effects on health in a broad variety of (patho-)physiological processes and also age-related diseases (103).

H_2S and sulfane sulfur species

The observed biological actions of H_2S do not solely result from H_2S in its primary biochemical state, but are also mediated by H_2S-derived sulfane sulfur species. Sulfane sulfur refers to uncharged sulfur atoms carrying six valence

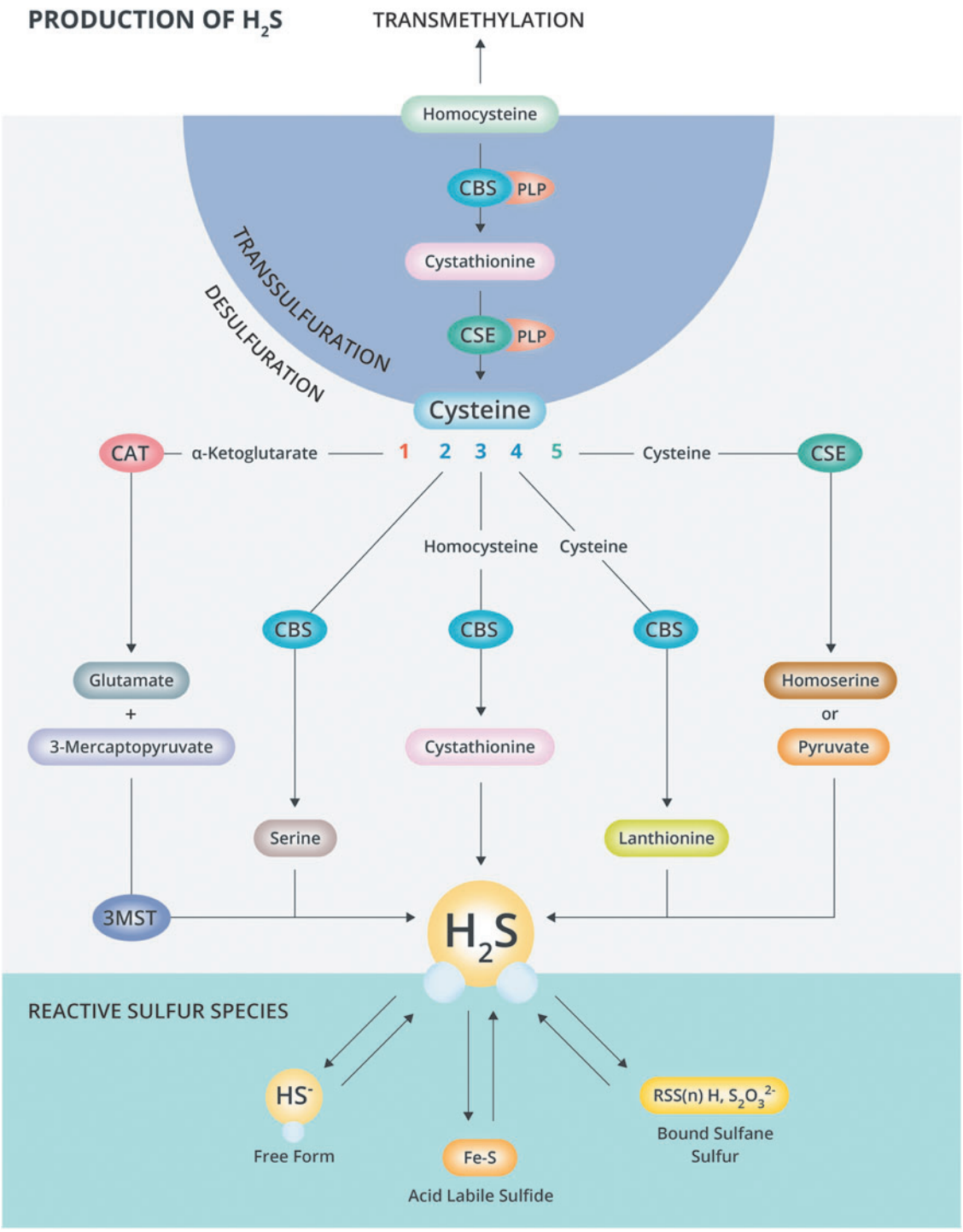

FIG. 1. Endogenous H_2S production in mammalian cells. Cysteine is the major precursor for H_2S synthesis. Cysteine is endogenously produced out of the transsulfuration pathway in which the dietary amino acids methionine, homocysteine, and cysteine serve as important substrates. Sulfur is transferred from homocysteine to cystathionine by the action of CBS, upon which CSE acts to generate cysteine. Homocysteine is the central substrate of the intersection of the transsulfuration pathway with the transmethylation pathway, as homocysteine can be converted back into methionine with the help of methyltetrahydrofolate and vitamin B_{12}. **(1)** Cysteine is converted by CAT into 3-MP, after which 3-MST exploits a sulfur atom from 3MP resulting in the formation of persulfide. In the presence of a reductant (*e.g.,* thioredoxin), H_2S is then released from the persulfide. **(2–4)** CBS is able to produce H_2S through converting cysteine into cystathionine and H_2S **(3)**, which runs analogously to the activity of this enzyme within the transsulfuration pathway. Alternatively, CBS can generate H_2S *via* a β-replacement reaction, which is accompanied by production of serine **(2)**. Another pathway constitutes the conversion of two cysteines by CBS to generate H_2S and the by-product lanthionine **(4)**. CSE catalyzes the conversion of cysteine to produce H_2S and several by-products, including pyruvate, NH_3, or homoserine **(5)**. Eventually, H_2S can give rise to a number of reactive sulfur species, including its free form (HS-), acid-labile sulfide (Fe-S), and bound sulfane sulfur compounds (*e.g.,* R-SS_n-H, $S_2O_3^-$). 3-MP, 3-mercaptopyruvate; 3-MST, 3-mercaptopyruvate sulfurtransferase; CAT, cysteine aminotransferase; CBS, cystathionine-β-synthase; CDO1, cysteine dioxygenase; CSAD, cysteine sulfinic acid decarboxylase; CSE, cystathionine gamma-lyase; GSH, glutathione; GSSG, glutathione disulfide; H_2S, hydrogen sulfide; NH_3, ammonia; PLP, pyridoxal-5′-phosphate.

electrons, and relevant sulfane sulfur compounds include persulfides (R-SS-H), polysulfides (R-S_n-SH or R-S-S_n-S-R), inorganic hydrogen polysulfides (H_2S_n, $n \geq 2$), and protein-bound elemental octasulfur (S_8) (49, 64, 75). H_2S shows a stepwise (1^- or 2^-) electron oxidation, forming thiyl radicals, disulfane (H_2S_2), and persulfides (56, 58). Among these sulfane sulfur species, H_2S_n are of particular interest as they are assumed to exert a number of H_2S-attributed (patho)physiological effects. H_2S_n may be endogenously produced *via* several enzymatic pathways. The main synthesis route is suggested to consist of the conversion of 3-mercaptopyruvate (3-MP) by 3-MST (50, 52). Another synthesis pathway that may contribute to H_2S_n production is facilitated by cysteinyl-tRNA synthetases (CARSs), which are able to effectively catalyze the formation of cysteine persulfide (Cys-SSH) and polysulfides (Cys-S_n-SH) when using cysteine as an enzymatic substrate (3). Third, H_2S_n can arise from the crosstalk between H_2S and NO, from which H_2S_n are generated together with nitroxyl (HNO), nitrososulfide (HSNO), and even $SSNO^-$ species (19, 51). Fourth, H_2S_n can be generated from H_2S in conjunction with haem proteins, in which cytosolic copper/zinc SOD may catalyze the H_2S oxidation and generate H_2S_n species (74). Sulfane sulfur compounds are often considered to act as a biochemical sulfide ''reservoir,'' releasing H_2S under reducing conditions and acting as a sink whenever H_2S concentrations rise (82).

Some of these individual reactive sulfur species (RSS) may exert a more potent effect than H_2S itself. For example, H_2S_n species have been demonstrated to activate transient receptor potential ankyrin 1 (TRPA1) channels in astrocytes to induce Ca^{2+} influx more potently than H_2S, a process thought to be mediated by persulfidation of two cysteine residues at the N-terminus of the TRPA1 channels (39, 53). In addition, endothelial nitric oxide synthase (NOS) and CSE within the vascular smooth muscle generate NO and H_2S that may interact and produce H_2S_n to activate protein kinase G (PKG)1α, which induces vasorelaxation (89). This, in turn, may be relevant in the context of COVID-19 where ''silent hypoxia'' (hypoxia in the absence of proportional signs of respiratory distress) coincides with (angiotensin II [Ang II]-induced) pulmonary vasoconstriction, giving rise to adverse profibrotic sequelae (11). Furthermore, endothelial dysfunction and microangiopathy are thought to be the principal pathophysiological mechanisms in COVID-19, leading to a shift of the vascular equilibrium to more vasoconstriction and resulting tissue ischemia, inflammation, and a procoagulant state, which might all be attenuated by vasorelaxant effects. Another striking example constitutes the S-persulfidation of protein cysteine residues by H_2S_n species, which has been suggested to largely contribute to the biological effects of H_2S_n and results in strong protective effects against oxidative stress, which in itself is heavily increased upon SARS-CoV-2 infection. H_2S_n species were demonstrated to activate the release of nuclear factor erythroid 2-related factor 2 (Nrf2) and its nuclear translocation to Kelch-like ECH-associated protein 1 (Keap1), which is persulfidated by H_2S_n, resulting in increased intracellular GSH and induction of HO-1 expression (55). Similarly, H_2S_n may regulate the activity of lipid phosphatase and tensin homologue (PTEN) by incorporation of sulfane sulfur into the active cysteine residue of PTEN, which is an important regulator of cell survival (37). These findings highlight that the balance between H_2S and its related sulfane sulfur compounds may be largely responsible for the aforementioned (patho-)physiological functions of H_2S, and, considering this, also the presumed effects of H_2S in the context of COVID-19.

H_2S and the reactive species interactome

A few years ago, the reactive species interactome (RSI) was introduced: a conceptual and integrative biological framework aimed to describe the chemical interactions among different types of reactive species, including reactive oxygen species (ROS), reactive nitrogen species, and RSS, and with their downstream biological targets such as cysteine-based thiols and metal centers (18). The RSI aims to describe not only the chemical interactions among reactive species but also the pathways that are involved in their generation through cellular intermediary metabolism, the transducing elements of redox regulation that modulate downstream intracellular targets, demonstrating adaptability to changes in metabolic demand, and the ability of sensing changes within the extracellular environment (12, 18). Interactions of reactive species with biological targets such as protein cysteine-based thiols can lead to short-term (*e.g.*, alterations in protein structure or activity) and longer term biological adaptations (*e.g.*, modification of targets involved in gene expression or regulation). The RSI is fueled by nutritional or therapeutic supply of reactive species precursors, including both organic (*e.g.*, L-arginine, L-methionine, and homocysteine) and inorganic (*e.g.*, oxygen, nitrite, and H_2S) compounds, as well as cofactors (*e.g.*, vitamin B_6 and vitamin B_{12}). In a biological system, the RSI may be viewed as a transducing and communicating modality that connects precursor availability and host/microbe cross talk with the extracellular redox state, which translates into effects on intracellular thiol targets.

In the context of the RSI, H_2S is an important precursor of RSS and its oxidation leads to the generation of sulfane sulfur species, for example, thiosulfate, H_2S_2, and sulfate. Although historically considered to be produced only under pathological circumstances, recent insights have indicated that RSS participate in a variety of physiological processes, controlling redox homeostasis, cellular signaling and metabolism, and mitochondrial function (79, 101).

H_2S As Antiviral Host Factor

A variety of preclinical studies have provided evidence of antiviral activity of H_2S. For instance, in case of respiratory syncytial virus (RSV) infections, H_2S demonstrated modulatory properties with regard to innate inflammatory responses and viral replication, both *in vitro* and *in vivo* (7, 8, 46, 60). Using methods to block the activity of H_2S-producing enzymes, animal CSE-knockout models, and H_2S donors, RSV replication and chemokine secretion were found to be significantly reduced by the modulation of transcription factors, including NF-κB and interferon regulatory factor-3 (IRF-3). Two main mechanisms were considered to play an important role in these actions. First, treatment with H_2S donors drastically reduced the cellular secretion of the pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) and the granulocyte colony-stimulating factor (G-CSF), as well as the release of chemokines IL-8,

regulated on activation, normal T cell expressed and secreted (RANTES), interferon-γ-inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1β from infected cells. Second, RSV-infected respiratory epithelial cells demonstrated a reduced ability to generate endogenous H_2S, but an enhanced propensity to degrade H_2S, indicating that viral infection, or the ensuing immune response, interferes with cellular H_2S homeostasis or H_2S depletion mechanisms (7, 8, 46, 60). Results from the aforementioned preclinical studies convincingly indicate both antiviral and anti-inflammatory properties of H_2S (Figs. 2 and 3). In the context of the current pandemic of COVID-19, preclinical studies elaborating on this were to be expected. Indeed, recent reports have recognized supportive evidence for a rationale to use H_2S-releasing molecules in COVID-19 (17, 28, 108).

Potential Enhancement of Endogenous H_2S Levels

Endogenous generation of H_2S may be triggered by various substances, among which NAC and taurine are most extensively studied (26). NAC is a potential H_2S-releasing donor as NAC-derived cysteine is desulfurated to H_2S, eventually leading to mitochondrial sulfane sulfur generation (29). Evidence from human cell lines demonstrates that both CSE and 3-MST are involved in NAC-induced H_2S production (29). Interestingly, an animal study using a maternal suramin-induced programmed hypertension model demonstrated that maternal NAC treatment protected male rat offspring against hypertension, which was associated with an increased protein level of 3-MST and H_2S synthesis in the kidney (92). Another rodent model showed similar results, this time involving NAC-induced enhancement of renal 3-MST and CBS (but not CSE) protein levels, as well as H_2S-releasing activity in offspring kidneys of hypertensive mothers (44). Taurine is a metabolite of cysteine and strongly boosts the CSE enzyme, and thereby contributes to increase the endogenous H_2S level in humans (90, 114). An overview of the endogenous taurine synthesis pathways is provided in Figure 4. Supplementation of taurine in prehypertensive individuals has been demonstrated to reduce blood pressure concurrent with an increase in plasma H_2S levels (90). Additional experimental studies in rodent models and isolated human mesenteric arteries showed that taurine treatment upregulated the expression of H_2S-synthesizing enzymes CSE and CBS (90, 117). Taurine is widely available, safe at higher doses, and cheap, making it an attractive compound to use as a treatment during this pandemic. In addition, taurolidine—available as an oral and intravenous antibacterial and anticancer drug—is a drug that can be degraded to taurine and water, but it is unknown whether this ultimately results in increased H_2S levels (47).

Potential Antiviral Actions of H_2S

Innate immunity

Components of the innate immune system act as first responders for detection and clearance of viral infections. However, many viral infections are characterized by evasion of the host innate immune response, resulting in viral cell entry and capture of the cellular translational machinery to enable viral replication. In particular, the type I interferon (IFN) response is typically bypassed, which usually promotes an antiviral immune state in both infected and neighboring cells, limiting viral replication and inducing apoptosis to protect the host from viral spread. Indeed, SARS-CoV-2 has evolved several mechanisms to evade the type I IFN

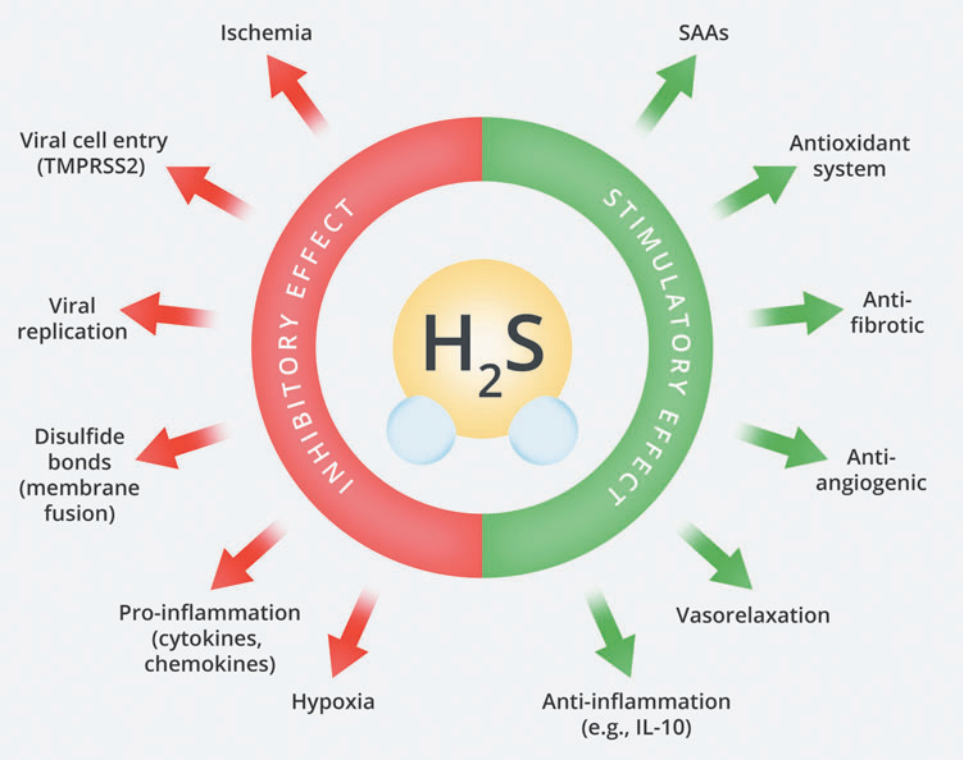

FIG. 2. Overview of stimulatory and inhibitory effects of H_2S in the context of COVID-19. COVID-19, coronavirus disease 2019.

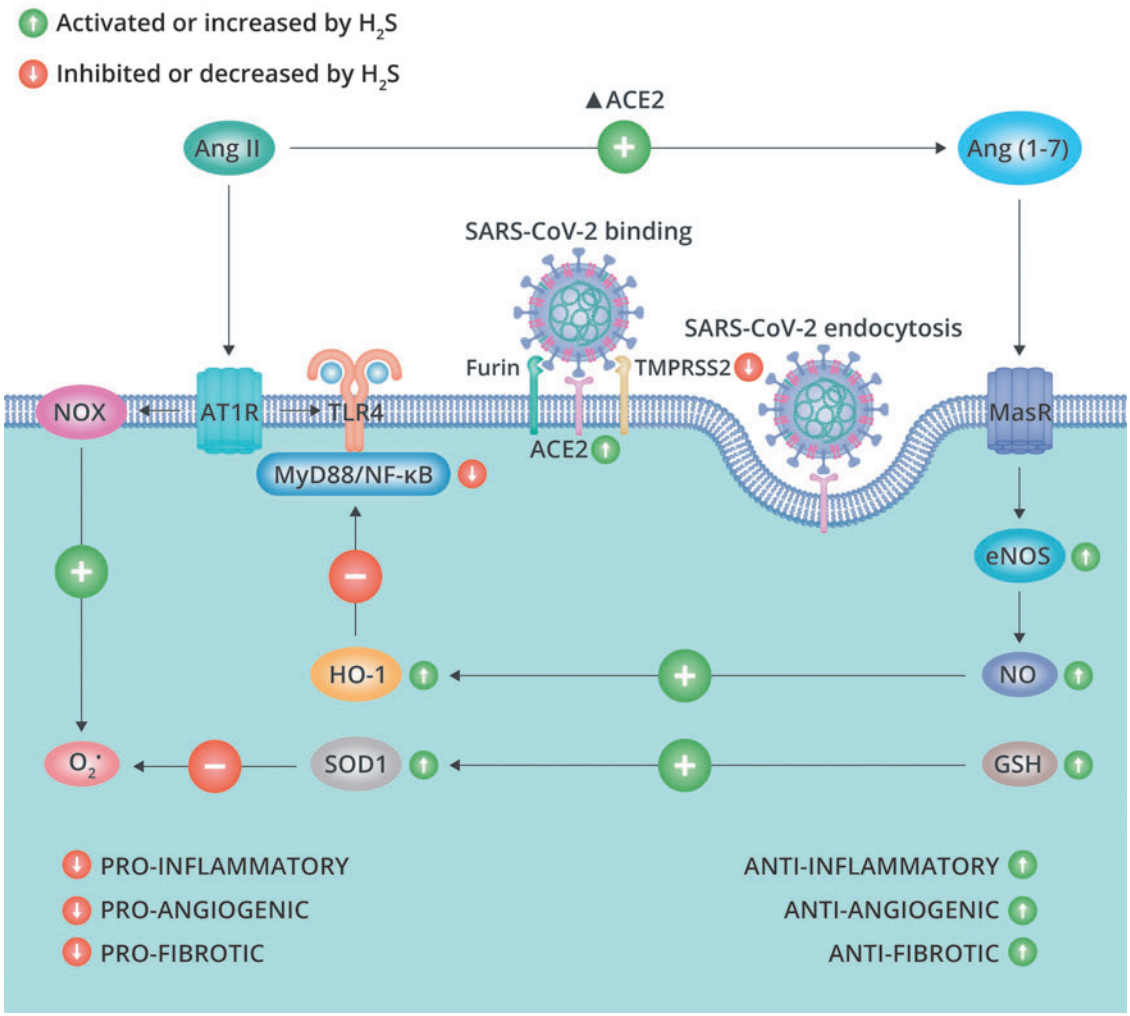

FIG. 3. Elaboration of stimulatory and inhibitory effects of H_2S in the context of COVID-19. H_2S inhibits TMPRSS2, a protease that amplifies SARS-CoV-2-entry *via* the endocytic pathway, as well as several proinflammatory cytokines *via* the TLR4/MyD88/NF-κB pathway and O_2^-. On the contrary, H_2S stimulates numerous anti-inflammatory mediators such as NO as well as its synthase (eNOS), GSH, HO-1, and SOD1. H_2S also increases the expression of ACE2, which converts proinflammatory Ang II into anti-inflammatory Ang (1–7). ACE2, angiotensin-converting enzyme 2; Ang II, angiotensin II; Ang (1–7), angiotensin (1–7); AT1R, angiotensin receptor type 1; eNOS, endothelial nitric oxide synthase; HO-1, heme oxygenase-1; MasR, Mas receptor; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; NOX, nicotinamide adenine dinucleotide phosphate oxidase; O_2^-, superoxide; SOD, superoxide dismutase; TLR4, toll-like receptor 4; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.

response. More specifically, multiple viral proteins (*e.g.*, open reading frame 6 [ORF6], ORF3b) have been shown to inhibit the innate immune system by suppression of IFN-I production and signaling (59, 107, 112). These mechanisms allow the virus to replicate and disseminate in the infected host. In contrast to most respiratory viruses, SARS-CoV-2 infection drives a lower antiviral transcriptional response, which is marked by low IFN-I and IFN-III levels and increased chemokine expression. This suggests that the innate antiviral defense against SARS-CoV-2 is imbalanced regarding the control of viral replication *versus* activation of the adaptive immune response. In this respect, treatment for COVID-19 should be less focused on the IFN response, but instead be directed to controlling inflammation (10).

Furthermore, significant dysregulation of monocytes and macrophages seems to be a key feature of severe COVID-19, apart from decreased levels of T-lymphocytes, an increased neutrophil-to-lymphocyte ratio (NLR), and depleted peripheral natural killer (NK)-cell counts (99). Interestingly, H_2S has been shown to be able to positively modulate concentrations of cytokines by reducing proinflammatory IL-6- and TNF-α-induced NF-κB activation, induced by TLR4 *via* the myeloid differentiation primary response 88 (MyD88)-dependent pathway at the cell membrane, while increasing the anti-inflammatory cytokine IL-10, induced by TLR4 after its endocytosis *via* the MyD88-independent pathway (62, 70) (Fig. 5). The ratio of IL-6:IL-10, named the Dublin/Boston score, is used as a tool to identify COVID-19 patients at risk of a poor outcome, by scoring the change between two IL-6:IL-10 ratio measurements taken 4 days apart, and an increased ratio is associated with worse disease prognosis (69). Furthermore, endogenous H_2S synthesis depends on the

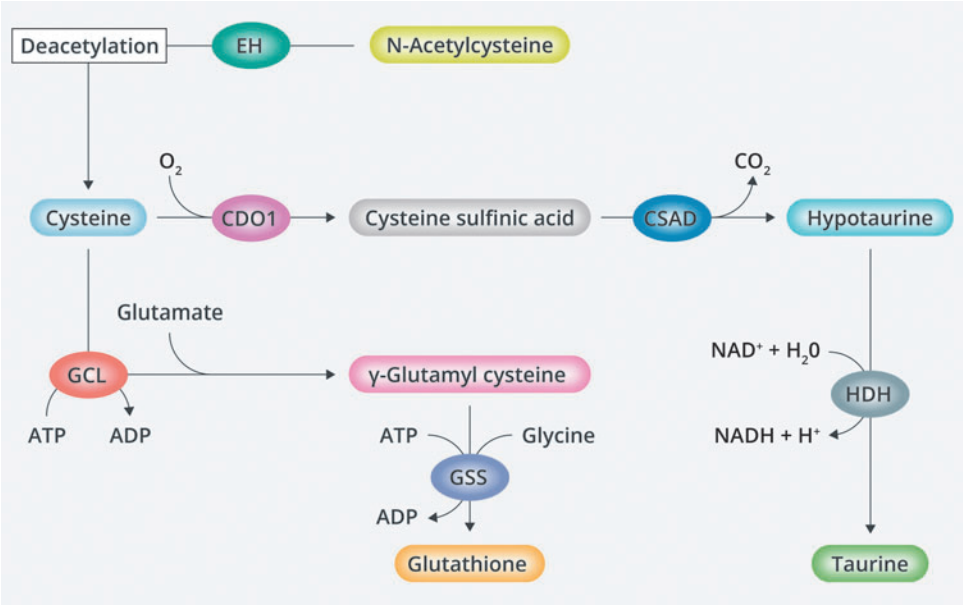

FIG. 4. Production of taurine and GSH from NAC. NAC is deacetylated into cysteine by the enzyme EH. CDO1 converts cysteine into cysteine sulfinic acid, which is further converted into hypotaurine by CSAD. Hypotaurine is oxidized by the action of HDH with NAD^+ and H_2O as additional substrates, yielding NADH, H^+, and taurine. GSH is synthesized from cysteine and glutamate *via* the production of γ-glutamylcysteine by the enzyme GCL and by GSS, using glycine as a cofactor. EH, epoxide hydrolase; GCL, gamma-glutamylcysteine ligase; GSS, glutathione synthetase; HDH, hypotaurine dehydrogenase; NAC, N-acetylcysteine; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced).

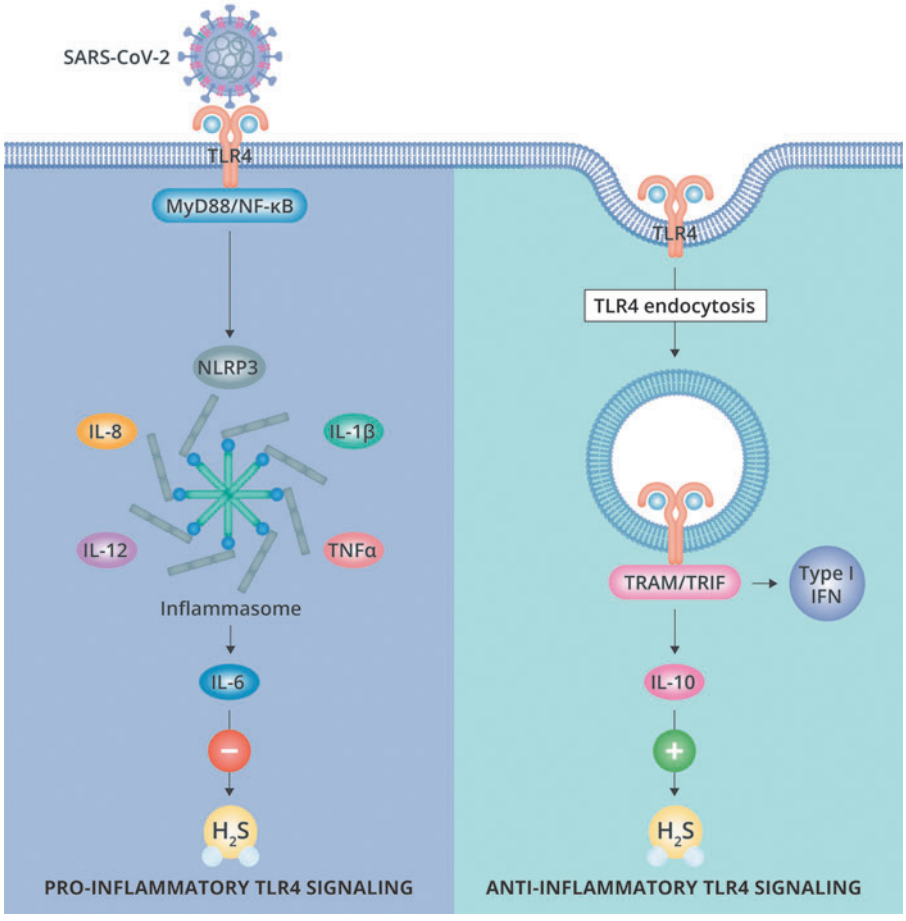

FIG. 5. Pro- and anti-inflammatory signaling by TLR4. SARS-CoV-2 infection triggers the consecutive activation of the proinflammatory TLR4/MyD88/NF-κB and the anti-inflammatory TLR4/TRAM/TRIF pathways. The first pathway inhibits H_2S synthesis and is associated with increased induction of IL-6. The successive anti-inflammatory pathway induces upregulation of IL-10, which activates H_2S production. This illustrates the rationale for the use of the Dublin/Boston score, the ratio IL-6:IL-10, as a tool to identify patients at risk of a poor outcome. IFN, interferon; IL, interleukin; NLR, neutrophil-to-lymphocyte ratio; NLRP3, NLR family pyrin domain containing 3; TNF-α, tumor necrosis factor-alpha; TRAM, translocation associated membrane protein; TRIF, TIR-domain-containing adapter-inducing interferon-β.

availability of IL-10. Previously, it was observed that IL-10-deficient mice could not produce H_2S, while administration of IL-10 restored H_2S synthetic capability (30). IL-10 is an important modulatory cytokine between innate and adaptive immunity and is produced by various immune cells, including T-lymphocytes. This means that increasing levels of IL-10 can be considered a manifestation of activation of adaptive immunity. IL-10 produced by effector Th1-lymphocytes limits the damage induced by excessive inflammation (98). Of note, activation of T-lymphocytes is also potentiated by H_2S (70).

Amino acid replenishment

SARS-CoV-2 infection has been demonstrated to cause rapid depletion of SAAs as a result of oxidative stress or inflammation-induced proteolysis (97). For instance, cysteine and taurine concentrations tended to decrease, especially in patients with moderate-to-high levels of IL-6. Simultaneously, increased levels of methionine sulfoxide and cystine, the oxidized dimer of cysteine, were observed, consistent with the possible explanation of increased oxidative stress. Taken together, this will reduce the net bioavailability of cysteine in patients with COVID-19, providing less substrate for endogenous production of H_2S. In this respect, NAC supplementation could be a therapeutic strategy to safely replenish cysteine depletion. Previously, NAC treatment has been demonstrated to successfully replenish whole-blood GSH as well as intracellular GSH of T-lymphocytes in human immunodeficiency virus (HIV)-infected patients who were depleted of SAAs (24, 27). In addition, a rapid increase in circulating cysteine levels has been observed within hours following NAC supplementation (88).

NLR family pyrin domain containing 3 inflammasome

The NLR family pyrin domain containing 3 (NLRP3) inflammasome is a multimeric protein complex, which plays a pivotal role in the innate host defense against viral infections and is activated by TLR4/MyD88/NF-κB signaling. It initiates an inflammatory type of cell death and leads to release of proinflammatory cytokines such as IL-1β and IL-18 (111). Aberrant activation of the NLRP3 inflammasome is implicated in a variety of human (inflammatory) diseases, including diabetes mellitus, infectious diseases, and neurodegenerative disorders. Hyperactivation of the NLRP3 inflammasome and release of downstream inflammatory mediators lead to pathological tissue injury during infection (20). In keeping with this, the SARS-CoV ORF3a accessory protein has been demonstrated to induce NLRP3 inflammasome activation. Both SARS-CoV and ORF3a were found to be potent activators of IL-1β gene transcription and protein maturation, which are required for NLRP3 inflammasome activation (87). Ultimately, the development of a proinflammatory cytokine storm is the net result, especially consisting of IL-1β, IL-6, and TNF-α. All these mediators play important roles in tissue inflammation as the driving force behind ARDS progression. Recently, the NLRP3 inflammasome has already been proposed as a potential therapeutic target in COVID-19. In addition to that, H_2S would be an interesting therapeutic candidate as it previously showed inhibition of NLRP3 inflammasome activation by suppressing the TLR4/MyD88/NF-κB pathway and reduction of the resulting cytokine production (15).

Angiotensin-converting enzyme 2 and the vascular compartment

Angiotensin-converting enzyme 2 (ACE2) has been unequivocally established as the functional host receptor for SARS-CoV-2 and is crucial for viral entry into cells (Fig. 6). Entry of SARS-CoV-2 into human cells primarily occurs in upper and lower respiratory epithelial cells (11, 91). Indeed, ACE2 is highly expressed on lung alveolar epithelial cells, providing the primary route of viral transmission. Further spread into the human host may be related to local ACE2 expression. The presence of ACE2 has been confirmed in vascular endothelial cells and smooth muscle cells in various human organs (38). SARS-CoV-2 infection leads to downregulation of ACE2 as this process entails internalization of the virus/receptor complex. However, this phenomenon is thought to compromise the physiological functions of ACE2 in the respiratory system. ACE2 is known to exert vasodilating, anti-inflammatory, antioxidant, and antifibrotic effects through cleavage of Ang II into angiotensin (1–7) [Ang (1–7)], which binds to the Mas receptor, inducing it to activate endothelial nitric oxide synthase (eNOS)-derived NO production. Ang II activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase leading to the formation of superoxide (O_2^-) radicals, which have proinflammatory effects opposing those of Ang (1–7)-induced NO. Therefore, loss of pulmonary function of ACE2 is associated with aggravation of ARDS or acute lung injury by disrupted regulation of the balance between pro- and anti-inflammatory signaling. Interestingly, H_2S may induce ACE2 upregulation and thereby attenuate pulmonary tissue injury (63). Recently, it was also hypothesized that H_2S may exhibit antiviral activity against SARS-CoV-2 by interfering with both ACE2 and transmembrane protease serine 2 (TMPRSS2) (108). In addition, H_2S potentially counteracts hypertension *via* two ACE2-dependent mechanisms. First, ACE2 induces conversion of Ang II into Ang (1–7), which activates eNOS to produce vasodilatory NO. Second, since Ang II enhances the degradation of CSE, preventing the synthesis of cysteine, H_2S, GSH, and taurine, conversion of Ang II will prevent CSE deficiency, leading to lowering of blood pressure and acting as a protective mechanism against organ damage within the experimental setting (6, 34).

Leukocyte-mediated inflammation can be modulated by both H_2S and NO, by attenuating proinflammatory cytokine-induced expression of leukocyte adhesion molecules and leukocyte infiltration (77, 93, 113). Considering the development of ARDS, a hyperinflammatory state, thrombosis, and associated ischemic events, this could have some relevance within the context of COVID-19 (33, 54, 100).

In addition, COVID-19 is associated with ''silent hypoxia.'' In the context of COVID-19, hypoxia is hypothesized to be induced by several mechanisms, such as pulmonary edema, loss of regulation of pulmonary perfusion, and intravascular formation of microthrombi (25). Acute hypoxemia enhances an inflammatory response and triggers counteracting responses to fully optimize oxygen consumption. ROS are excessively produced upon hypoxia, and act as second messengers of these responses (40, 94).

H_2S has been identified as an excitatory mediator of hypoxic sensing in the carotid bodies (106). The carotid body expresses mRNA for both CBS and CSE and hypoxia

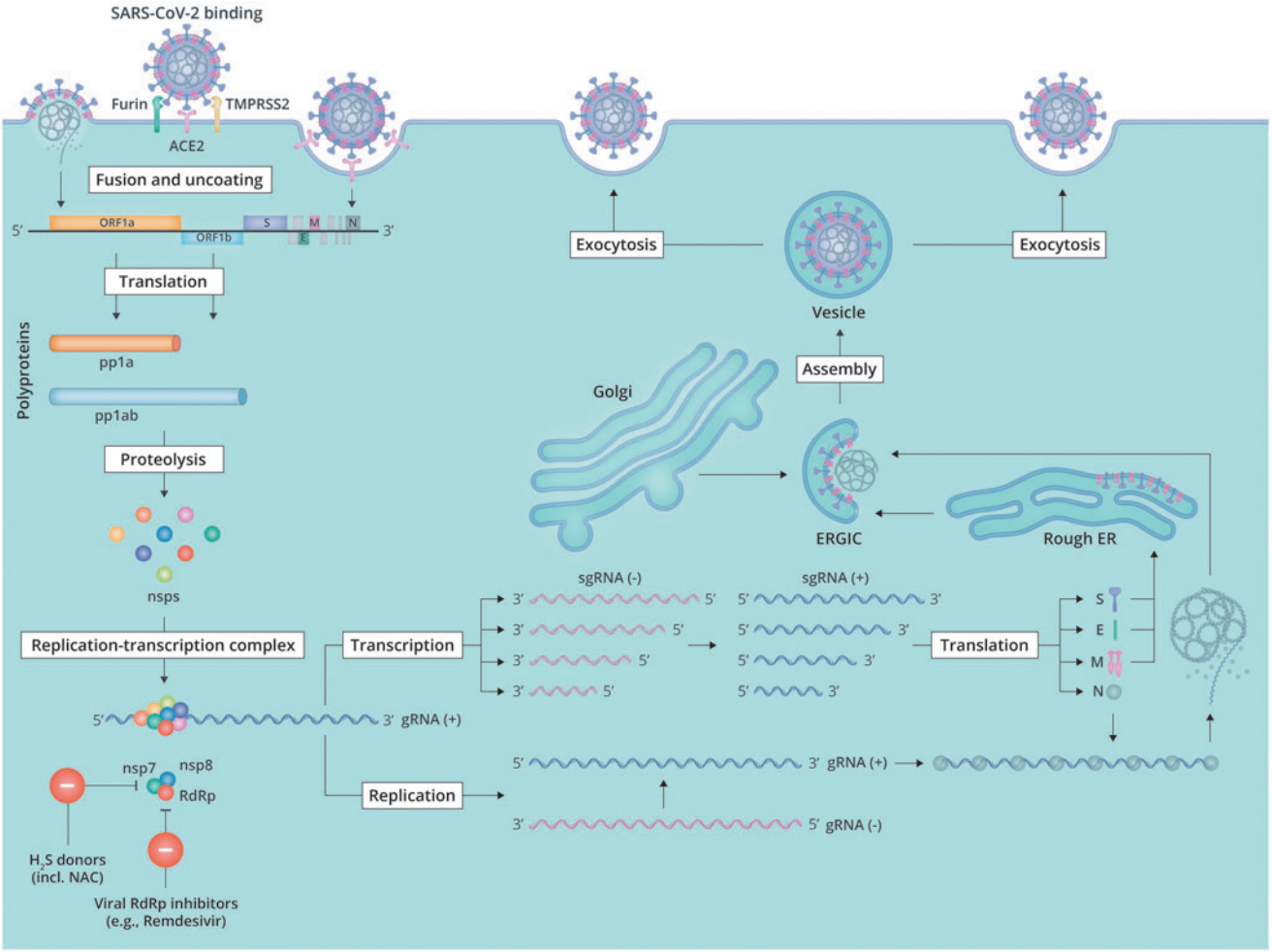

FIG. 6. The life cycle of SARS-CoV-2 in host cells. Following binding of S glycoprotein to ACE2 and other host factors (such as TMPRSS2), SARS-CoV-2 enters the host cell either *via* endocytosis or direct fusion of the viral envelope with the endosomal membrane, or *via* direct fusion with the cellular membrane. Then, viral uncoating with subsequent release of genomic RNA leads to primary translation of two large ORFs, namely ORF1a and ORF1b. The resultant polyproteins pp1a and pp1ab are then degraded into individual nsps, which form the RCT to produce more RNA. The complex of RdRp with nsp7 and nsp8 plays a key role in replication of gRNA and transcription of sgRNA, and forms a therapeutic target for RdRp inhibitors (such as remdesivir) and H_2S donors (such as NAC). This is followed by translation of structural proteins that translocate to the ER and later ERGIC, where they meet with N-surrounded gRNA and assemble into new viral particles by budding. Eventually, virions are released from the infected cell *via* exocytosis. E, envelope; ER, endoplasmic reticulum; ERGIC, endoplasmic-reticulum-Golgi intermediate compartment; gRNA(+), positive stranded genomic ribonucleic acid; gRNA(-), negative stranded genomic ribonucleic acid; M, membrane; N, nucleocapsid; nsp, nonstructural protein; ORF1a, open reading frame 1a; ORF1b, open reading frame 1b; pp1a, polyprotein 1a; pp1ab, polyprotein 1ab; RCT, randomized-controlled trial; RdRp, RNA-dependent RNA polymerase; S, spike; sgRNA, subgenomic ribonucleic acid.

increases local H_2S generation. At a cellular level, H_2S can be oxidized during hypoxia, when a sulfide:quinone reductase feeds the mitochondrial respiratory chain with the hydrogen atoms of sulfide, directing reduction of malate to succinate by reversing mitochondrial complex II (35). Relevant to this mechanism is the requirement of CSE translocation to the mitochondria to support energy (adenosine triphosphate [ATP]) production (31). Furthermore, CBS may partially compensate for the limited mitochondrial translocation of CSE in cells expressing CBS, as hypoxia leads to mitochondrial accumulation of CBS by failure of Lon protease to degrade CBS (a process that is determined by the oxygenation status of the haem group contained in the CBS protein) (96). This mechanism consumes H_2S, which might lead to a deficiency, while on the contrary, H_2S has higher stability and is produced in higher amounts during hypoxia (2, 66). As such, H_2S may function as energy substrate to sustain ATP production under hypoxic stress conditions.

Oxidative stress that is induced by hypoxia increases the ratio of oxidized to reduced GSH (glutathione disulfide [GSSG]:GSH). GSSG induces S-glutathionylation of endothelial NOS, causing eNOS uncoupling, which leads this enzyme to produce O_2^- instead of NO (78). Treatment with NAC results in increased levels of GSH, which may prevent eNOS uncoupling by decreasing the GSSG:GSH ratio (Fig. 7).

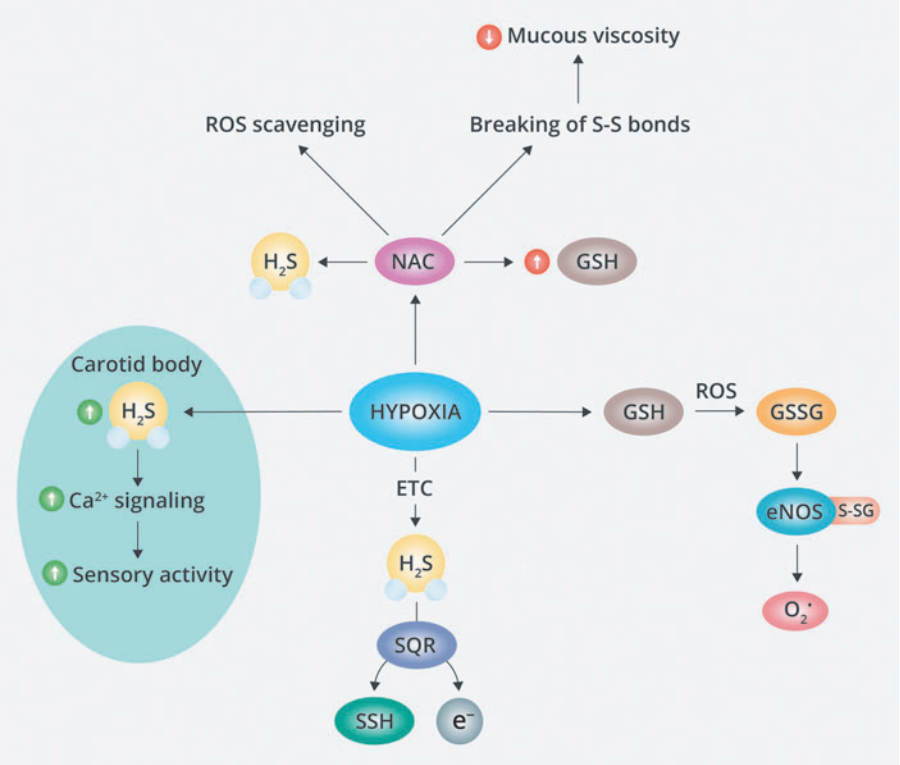

FIG. 7. Interrelationships between NAC, H_2S, and hypoxia. Hypoxia leads to an increase in local H_2S production within the carotid bodies, which functions as an excitatory mediator of hypoxic sensing through increased Ca^{2+} signaling. Under hypoxic conditions, oxidation of H_2S fuels the mitochondrial respiratory chain by shuttling electrons to the mitochondrial quinone pool. NAC treatment has a number of antioxidant effects, including direct ROS-scavenging effects through its intrinsic thiol activity, increasing GSH levels, breaking disulfide bonds, which results in decreased mucous viscosity, but it can also be desulfurated to H_2S. Increased levels of GSH may prevent eNOS uncoupling by decreasing the GSSG:GSH ratio, which is elevated in circumstances of hypoxia-mediated oxidative stress and leads to increased S-glutathionylation of eNOS, causing this enzyme to produce O_2^- radicals instead of NO. ETC, electron transport chain; ROS, reactive oxygen species; SQR, sulfide:quinone reductase.

Pharmacological Aspects and Therapeutic Potential of NAC

Pharmacology of NAC and potential antiviral effects

NAC is one of the most widely used antioxidants in scientific research, being frequently applied in both *in vitro* studies and *in vivo* studies. Typically, observed effects of NAC in different settings are associated with a decrease of ROS. Despite its widespread actions, the exact antioxidant mechanism has a multidimensional character and is more complex than it may seem at first glance. First, NAC is assumed to directly scavenge reactive species by its own thiol group. However, based on the kinetics of redox reactions with common physiological oxidants such as H_2O_2 and O_2^-, the NAC-thiol group has low intrinsic reactivity toward oxidants (9). Second, NAC is assumed to act as a cysteine prodrug to increase GSH biosynthesis. NAC easily penetrates cells where it is deacetylated to yield L-cysteine, thereby promoting GSH synthesis. Through this mechanism, situations of decreased GSH bioavailability may benefit from treatment with NAC, as it primarily works in the extracellular environment and acts as precursor for intracellular GSH. For instance, in the clinical case of acetaminophen intoxication, NAC is frequently applied to restore GSH depletion in the liver (36). Accordingly, all its intracellular effects are mediated by GSH replenishment (23). Third, NAC has been demonstrated to exert an H_2S-generating effect (16, 29). NAC can be desulfurated to H_2S, which in turn may be oxidized within mitochondria to generate sulfane sulfur species. These species may contribute to the antioxidant and cytoprotective effects as are observed for NAC. *Via* this mechanism, NAC is assumed to provide a relatively fast antioxidant effect as the actual mediators of sulfane sulfur species production—3-MST and sulfide:quinone oxidoreductase—to ensure rapid occurrence of this reaction.

NAC can be administered in several ways, including orally, intravenously, and by nebulization. NAC has mucolytic properties in the airways by breaking disulfide bonds residing in the mucus (76). Disulfide bonds connect two cysteine residues and provide proteins with important structural and functional characteristics. The ability to break disulfide bonds may be important in the context of SARS-CoV-2 infection, as disulfide bonds may play a role in viral fusion with the host cellular membranes. Enveloped coronaviruses have a peak S2 domain, which is required for membrane fusion activation and is flanked by cysteine residues C822 and C833, which are part of the SARS-CoV-2 fusion protein (FP). FPs consist of two subdomains, FP1, lying immediately downstream of the S2′ cleavage site, and FP2, a region further downstream of FP1. The two cysteines C822 and C833 are

considered to form an internal disulfide bond, which provides the domain with a loop structure. However, it is questionable whether these disulfide bonds are able to resist local H_2S or NAC in the membrane fusion region (65). Previously, it has been investigated if such a disulfide bond could play a role in FP2-mediated membrane ordering (57). In this study, the authors demonstrated that 5 m*M* dithiothreitol (DTT), a strong reducing agent that is able to remove disulfide bonds, completely abrogated the membrane-ordering effect of FP2.

Apart from cell entry of SARS-CoV-2, H_2S may also theoretically exert an antiviral effect within the cytosol. The RNA-dependent RNA polymerase (RdRp, or nonstructural protein [nsp] 12), the major component of viral replication and transcription machinery, is a therapeutic target in SARS-CoV-2 infection. For example, RdRp is assumed to be the primary target of the antiviral drug remdesivir. RdRp functions in a complex with two cofactors, nsp7 and nsp8, and catalyzes the synthesis of viral RNA. Recently, a specific substructure of the SARS-CoV-2 RdRp domain, which is the N-terminal β-hairpin, has been identified (32). This structure inserts into the groove clamped by the nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain architecture of SARS-CoV-2, as well as the palm subdomain within the RdRp. In determining the structure of the SARS-CoV-2 RdRp, in complex with nsp7 and nsp8, two different protocols using DTT were adopted. In the absence of DTT, it was observed that cysteines C301–C306 and C487–C645 formed disulfide bonds. However, in the presence of DTT, chelated zinc ions were present, similar to the location of these zinc ions as observed in SARS-CoV-1. Based on these findings, it is questionable whether the disulfide bonds residing in the nsp12-nsp7-nsp8 complex of SARS-CoV-2 are able to resist the potential local effects of NAC or other H_2S donors. In this respect, it is important to note that H_2S functions as a gasotransmitter, and is thus not bothered by biological membranes.

Timing of treatment

The clinical course of COVID-19 is characterized by several disease stages. Initially, in the early stage of infection, viremia arises, followed by a pulmonary phase that consists of an acute clinical presentation of pneumonia. After this pulmonary phase, most patients gradually recover. However, a small subset of patients progresses to a more severe disease that may consist of ARDS, acute kidney injury, or even MOF, requiring intensive care unit (ICU) admission. Risk factors for COVID-19 (*i.e.*, obesity, patient comorbidity) as well as factors such as immune function and ACE/ACE2 balance may determine who progresses to this severe disease stage and who will not. Importantly, each phase of the disease demands its own treatment regimen, ranging from antiviral therapy (*e.g.*, blocking viral entry or inhibition of viral replication) in the initial stage, to anti-inflammatory, antioxidant, and antithrombotic therapy in the later stages of the disease.

Considering this, the timing of treatment with NAC or H_2S donors should be synchronized with concurrent medical treatments (86). In a recent double-blind, placebo-controlled RCT, high-dose NAC was provided to patients with COVID-19 in a fairly late stage of the disease, without subsequent addition of steroids, and it did not show any clinical benefit (4). As soon as the disease reaches the host inflammatory response phase and symptoms are worsening (usually around 7–10 days), one could consider the addition of dexamethasone to NAC as combined anti-inflammatory and antioxidant therapy to reverse the progressive host inflammatory response. On the contrary, as SARS-CoV-2 infection is characterized by a high viral load at the onset of clinical symptoms, one could consider the potential antiviral effects of NAC. However, it should be noted that chronic intake of NAC or H_2S donors is not recommended. Based on current knowledge, it would be most advisable to start treatment with an incipient viral infection or a nearby viral threat, or just at the beginning of the host inflammatory response phase. Based on the endogenous property of taurine to inhibit TLR4/MyD88-signaling and activation of the NLRP3 inflammasome, as well as its antioxidant activity and its capacity to increase the H_2S-synthesizing enzyme CSE, this compound may be an effective disease modulator during all stages of COVID-19.

Nebulization

Nebulization in the context of COVID-19 has initially been criticized because of the risk of viral contamination from aerosol or droplets. Nevertheless, nebulization is not contraindicated as long as sufficient precautions are being taken. Safe nebulization therapy with a mesh nebulizer could be achieved with negative chamber pressure or curtain temporary negative pressure isolation. Using appropriate personal protective equipment, the specific medication can be safely delivered onto virus-infected airway epithelial cells (68, 86). By adding sodium bicarbonate (4.2%) to the nebulizing solution, which is highly virucidal and commonly used in the food industry, viral infectivity can be significantly reduced (67). Safety of this nebulizing solution has been proved for over 30 years and its recipe was originally described in 1948 (22).

Clinical Relevance of H_2S and NAC in the Context of COVID-19

H_2S as prognostic factor in severe COVID-19

Recently, a clinical study was published that investigated serum H_2S levels in relation to disease severity and disease outcome in 74 patients who were hospitalized because of COVID-19 pneumonia (83). Baseline H_2S levels demonstrated a high discriminative performance with regard to pneumonia-associated survivors and nonsurvivors, with 80% sensitivity and 73.4% specificity. In a similar manner, a decrease in H_2S levels during hospitalization was associated with a higher mortality rate. In addition, serum H_2S levels were negatively correlated to IL-6, procalcitonin, and CRP, indicating an increased utilization of H_2S among patients with a higher inflammatory disease state. Findings from this study led to consider exogenous H_2S supplementation as a potential therapeutic strategy in patients with COVID-19. However, these findings should be interpreted with caution as it is questionable whether H_2S can be reliably measured in serum.

Case reports highlighting the therapeutic potential of NAC in COVID-19

A case series from New York reported on 10 consecutive patients having severe COVID-19 who demonstrated marked clinical and biochemical improvement upon intravenous administration of NAC (45). One patient with glucose-6-phosphate deficiency (G6PD), who is especially predisposed to hemolysis induction, was affected by severe COVID-19 and initially treated with hydroxychloroquine. This patient was respirator-dependent and his respiratory status deteriorated quickly, requiring intubation and maximum ventilation, and eventually veno-venous extracorporeal membrane oxygenator treatment was initiated. Soon after, however, he was described to have remarkable clinical improvement upon intravenous NAC treatment with complete reversal of hydroxychloroquine-induced severe hemolysis and decreased levels of inflammatory markers. Subsequently, nine additional patients without G6PD deficiency but with severe, respirator-dependent COVID-19 were treated with intravenous NAC. Again, similar benefits were observed with evident clinical improvement and reduced levels of inflammatory markers.

Another case report from New York described two patients with a history of Lyme disease and tick-borne coinfections who suffered from severe cough and dyspnea and had radiological findings compatible with COVID-19-associated pneumonia (43). Both patients demonstrated immediate clinical improvement after treatment with high dose oral and/or intravenous GSH treatment combined with NAC and antioxidant (alpha-lipoic acid, vitamin C) administration. Repeated treatment with GSH continued to improve their respiratory symptoms.

In line with the above, another case report from the United States described one critically ill patient with COVID-19 who was successfully treated with intravenous administration of high-dose NAC with concurrent treatment of low-dose hydroxychloroquine (81). There was noticeable clinical improvement and a marked decrease of several inflammatory markers, including ferritin, CRP, and lactic acid levels. Interestingly, thrombo-embolic complications of COVID-19 were later observed, as the patient developed pulmonary embolism and deep venous thrombosis, which eventually resolved upon thrombolysis and heparinization.

Trials in progress investigating the therapeutic potential of NAC in COVID-19

Currently, several clinical trials are ongoing to investigate the therapeutic potential of NAC or H_2S precursors in improving disease status in patients with COVID-19 (Table 1). In the aforementioned double-blind, placebo-controlled RCT testing high-dose NAC in patients with COVID-19, NAC did not demonstrate any clinical benefit, however, it was administered only very late in the disease course, at least later than 7–10 days after the onset of disease symptoms (4). Further studies are warranted to further determine the most appropriate timing of NAC supplementation in various cases of COVID-19.

Discussion

In this review, we describe the potential role of H_2S as a fundamental host defense factor against SARS-CoV-2 infection. Accumulating evidence indicates that H_2S might be acting as an antiviral host factor in patients with COVID-19, whereas it also seems to have a multifaceted mode of action in targeting SARS-CoV-2 infection. Given the fact that endogenous H_2S production can be therapeutically augmented by administration of NAC, taurine, or related components,

Table 1. Overview of Currently Ongoing Trials Investigating N-Acetylcysteine As Potential Therapeutic in Patients with Coronavirus Disease 2019

Location and NCT No.	*Design*	*Patients*	*Intervention*
New York, United States, NCT04374461	Phase II, single-center study, two treatment arms: mechanically ventilated/critically ill *versus* noncritically ill and nonmechanically ventilated	Severe or critically ill patients, estimated enrollment of 84 patients	Intravenous NAC 6 g/day
South Carolina, United States, NCT04545008	Phase I, single-center study, several treatment arms with different (combinations of) dosages	Outpatient setting, newly diagnosed SARS-CoV-2 infection not requiring hospitalization, estimated enrollment of 42 patients	Oral NAC 600–1800 mg three times daily with or without oral famotidine 20–80 three times daily
Cambridge, United States, NCT04419025	Phase II, multicenter study, randomized parallel-group assignment	Mild-to-moderate disease, both inpatients and outpatients, estimated enrollment of 200 patients	NAC 25 mg/kg every 4 h until discharge, 1200 mg twice a day until 1 week post-discharge, or 2400 mg once a day for 1 week, then 1200 mg twice a day for 2 weeks
United States, NCT04458298	Phase II, double-blind, placebo-controlled study, randomized sequential assignment	Severe disease, estimated enrollment of 24 patients	OP-101 (dendrimer NAC), single infusion of 2, 4, or 8 mg/kg, or a matching placebo

NAC, N-acetylcysteine; NCT, National Clinical Trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

H_2S becomes an attractive compound with a huge potential to provide clinical benefit. Considering the *in vitro*, *in vivo*, preclinical, and clinical studies reviewed above, H_2S emerges as an important host factor against viral infections. In line, systemic H_2S levels have been attributed the role of a prognostic marker in the case of severe COVID-19-associated pneumonia.

Characteristically, the clinical course of COVID-19 is usually mild in $\sim$80% of infected individuals, whereas around 10%–15% of people infected progress to a more severe disease, requiring hospitalization for supportive respiratory care, or even ICU admission. Since the majority of infected individuals are asymptomatic or only experience mild symptoms, there must be a vast amount of antiviral host factors that could explain the heterogeneity in disease course. For instance, some complicating risk factors have frequently been addressed that may cause the potential role of H_2S to differ among individuals. Advanced age has been recognized as one of the strongest predictors for severe COVID-19 and is an established risk factor for the disease, as is evident from the high rates of complications and case fatalities among older patients. Many biological explanations to this increased risk of contracting severe COVID-19 have been proposed, including an age-related decline in immune function (''immunosenescence''), a chronic state of metabolic systemic inflammation (''inflammaging''), or changes in ACE2 expression and activity (11, 71). Considering NAC and H_2S, there is also an age-related decline in the efficiency of GSH synthesis, as GSH tissue levels tend to decrease with age. This aging-associated GSH deficit can be corrected with NAC supplementation (84). As such, by replenishing the endogenous GSH stock, there would be sufficient cysteine precursors to endogenously generate H_2S. There is also a sex-associated predisposition to COVID-19, with men being more prone to develop severe disease compared with women. Again, there are many possible biological explanations to this sex-specific risk stratification, including differences in chromosomal ACE2 expression, immune system regulation, renin/angiotensin/aldosterone system (RAAS) regulation, and lifestyle factors. Relating to H_2S physiology, estrogens boost the expression of CSE within the vasculature and thus promote the generation of H_2S, delivering more favorable effects of H_2S to reproductive females. Finally, comorbidity has been established as a significant risk factor for severe COVID-19, including, among others, cardiovascular disease, chronic respiratory disease, diabetes mellitus, obesity, and IMIDs. Many of these conditions have also been associated with a decreased H_2S-generating capacity, especially hypertension, diabetes mellitus, and obesity (34, 48, 102).

The possibility that NAC could act as an endogenous modulator of H_2S production was first demonstrated within the context of leukocyte-mediated inflammation (113). In this study, the observed anti-inflammatory and antioxidant effects of NAC could be reversed by an inhibitor of CSE, proving that effects were at least partially mediated by H_2S generation. Two years ago, another study confirmed the H_2S- and sulfane sulfur species-generating effect of NAC to be responsible for the immediate antioxidant and cytoprotective effects of NAC (29). Hitherto, these findings have been rather neglected in literature as the assumed antioxidant activity (by providing cysteine to promote GSH biosynthesis) continues to be underscored as the main mode of action, while the associated H_2S-release provided by NAC has been given less prominence. Similarly, studies proposing NAC as a potential therapeutic agent in COVID-19 differently explain its effects by either emphasizing the potentiation of endogenous H_2S levels or focusing on the main antioxidant activity of the drug (23, 80, 108). For the most part, potential beneficial effects of NAC will be mediated by similar processes, as both pathways signal *via* oxidation reactions with protein cysteine sulfur, eventually producing identical effector responses (74). As the H_2S-release by NAC supplementation could be an important mode of action of NAC in COVID-19, it would be important to know where H_2S is being synthesized in patients with COVID-19 (*e.g.*, there could be reduced local expression of CSE in the lungs, whereas it might be preserved in other organs) and how this relates to the efficacy of NAC treatment.

H_2S could potentially act multitargeted against SARS-CoV-2, including actions against viral cell entry, viral replication, oxidative stress, and the escalation of inflammation leading to a cytokine storm. All these targets align with clinical trial-explored drug targets for COVID-19. However, it is important to further study the dynamics of the antiviral and anti-inflammatory actions of H_2S. Available evidence as reviewed above indicates that SARS-CoV-2-infection quickly leads to a compromised innate immunity and depletion of SAAs, the latter of which rapidly decreases endogenous H_2S levels. Conversely, the generation of H_2S also seems to be a fast-acting, dynamic process, as supported by the observed short-term clinical effects. From a safety standpoint, H_2S could likely be immediately degraded once it is either positively or negatively modulated. Indeed, a fast-acting, high dose of NAC appeared to be sufficient for supplementation, according to the clinical reports of NAC administration in patients with severe COVID-19. Safety of NAC administration is further supported by relatively low serum and tissue H_2S concentrations measured in *in vivo* studies *versus* relatively high and rapidly increasing concentrations observed in various *in vitro* studies upon administration of artificial H_2S donors. Thus, modulation of endogenous H_2S production is apparently safe, but further intensive dose finding studies are warranted to confirm this, especially when NAC is administered in the higher dose range. Taurine may also be considered a disease-modifying treatment, based on its capacity to increase H_2S production, as well as its own antioxidant actions.

Another factor that deserves consideration when administering NAC in patients with COVID-19 is the timing of treatment. As outlined earlier, the disease course of COVID-19 is characterized by several stages, each demanding its own treatment regimen. The ratio of the viral load to the host inflammatory response is believed to determine the specific treatment. Starting antiviral therapy in a timely manner while containing the host inflammatory response at already an early stage would be most preferable in this respect. As such, it would theoretically be possible to prevent worsening of the disease, resulting in severe tissue damage during the inflammatory phase (86). Preferably, antiviral NAC therapy (oral or nebulizer) would be introduced at an early stage of the disease, while steroids (*e.g.*, dexamethasone) could be complementary to NAC at the later stage of the host's inflammatory response (13).

NAC could be used as a repurposed medication as it is very safe, widely available, cheap, and rarely presents with side

effects, making it very feasible to conduct RCTs to test its clinical effects (Table 1). Only one single drug will be highly unlikely to produce sufficient clinical benefit in patients with COVID-19. Therefore, a rationalized, evidence-based combination of different drugs may prove necessary to attenuate disease severity.

Conclusion

H_2S constitutes a versatile and modifiable host factor in patients with COVID-19. Since the endogenous generation of H_2S is amenable to therapeutic modulation by administration of H_2S donors, this may become an additive therapeutic strategy in COVID-19. Evidence supports both antioxidant and antiviral effects of H_2S, which is further sustained by the successful outcomes of a variety of case studies describing the role of H_2S in severe COVID-19 pneumonia. In addition, milder phases of COVID-19 may be attenuated by H_2S as an antiviral host factor, and it may potentially be used as preventive antioxidant supplementation. Nevertheless, future studies are required to further examine the effectiveness and applicability of NAC or taurine supplementation to promote endogenous H_2S production in patients with COVID-19. More clinical studies are warranted that focus on the optimal dosages and timing of treatment, as well as those reporting follow-up data of patients treated with H_2S donors.

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Abbreviations Used

3-MST = 3-mercaptopyruvate sulfurtransferase
ACE2 = angiotensin-converting enzyme 2
Ang (1–7) = angiotensin (1–7)
Ang II = angiotensin II
ARDS = acute respiratory distress syndrome
AT1R = angiotensin receptor type 1
ATP = adenosine triphosphate
CBS = cystathionine-β-synthase
CDO1 = cysteine dioxygenase
COVID-19 = coronavirus disease 2019
CRP = C-reactive protein
CSAD = cysteine sulfinic acid decarboxylase
CSE = cystathionine gamma-lyase
DTT = dithiothreitol
E = envelope
EH = epoxide hydrolase
eNOS = endothelial nitric oxide synthase
ER = endoplasmic reticulum
ERGIC = endoplasmic-reticulum-Golgi intermediate compartment
ETC = electron transport chain
FP = fusion protein
G6PD = glucose-6-phosphate deficiency
GCL = gamma-glutamylcysteine ligase
gRNA(+) = positive stranded genomic ribonucleic acid
gRNA(−) = negative stranded genomic ribonucleic acid
GSH = glutathione
GSS = glutathione synthetase
GSSG = glutathione disulfide
H_2S = hydrogen sulfide
H_2S_2 = disulfane
H_2S_n = hydrogen polysulfides
HDH = hypotaurine dehydrogenase
HO-1 = heme oxygenase-1
ICU = intensive care unit
IFN = interferon
IL = interleukin
IMID = immune-mediated inflammatory disease
M = membrane
MasR = Mas receptor
MOF = multiorgan failure
MyD88 = myeloid differentiation primary response 88
N = nucleocapsid
NAC = N-acetylcysteine
NCT = National Clinical Trial
NAD^+ = nicotinamide adenine dinucleotide (oxidized)
NADH = nicotinamide adenine dinucleotide (reduced)
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
NH_3 = ammonia
NLR = neutrophil-to-lymphocyte ratio
NLRP3 = NLR family pyrin domain containing 3
NO = nitric oxide
NOS = nitric oxide synthase
NOX = nicotinamide adenine dinucleotide phosphate oxidase
nsp = nonstructural protein

Abbreviations Used (Cont.)

O_2^- = superoxide
ORF = open reading frame
PLP = pyridoxal-5′-phosphate
pp1a = polyprotein 1a
pp1ab = polyprotein 1ab
PTEN = phosphatase and tensin homologue
RCT = randomized-controlled trial
RdRp = RNA-dependent RNA polymerase
ROS = reactive oxygen species
RSI = reactive species interactome
RSS = reactive sulfur species
RSV = respiratory syncytial virus
S = spike
SAA = sulfur-containing amino acid
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
sgRNA = subgenomic ribonucleic acid
SOD = superoxide dismutase
SQR = sulfide:quinone reductase
TLR = toll-like receptor
TMPRSS2 = transmembrane protease serine 2
TNF-α = tumor necrosis factor-alpha
TRAM = translocation-associated membrane protein
TRIF = TIR-domain-containing adapter-inducing interferon-β
TRPA1 = transient receptor potential ankyrin 1