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Invited Opinion

H_2S AS A THERAPEUTIC ADJUVANT AGAINST COVID-19: WHY AND HOW?

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Recently, H_2S has been proposed as a potential therapy for patients with SARS-CoV-2 (COVID-19) pneumonia (1), and in this context, its effects have been attributed to various mechanisms:

1. It is well established that SARS-CoV-2 enters the cell *via* docking to the membrane-bound angiotensin-converting enzyme2 (ACE2) after cleavage of one of its surface proteins by the host's transmembrane protease serine 2 (TMPRSS2). In fact, both the severity (2) and extra-pulmonary manifestations (3) of the SARS-CoV-2 disease have been associated with variable ACE2 and/or TMPRSS2 activities. Consequently, H_2S-related reduction of their activity could be therapeutically relevant (4).
2. Besides the blockade of virus entry into the cell, H_2S may act *via* inhibition of virus replication as demonstrated in other RNA viruses (5): the slow-releasing H_2S donor GYY4137 attenuated alveolar epithelial cell pro-inflammatory cytokine release due to reduced virus replication, while both genetic deletion (6) and pharmacological inhibition (7) of cystathionine-γ-lyase (CSE), one of the major H_2S-producing enzymes, exerted the opposite effect.
3. Oxidative stress (8, 9) resulting from glutathione (GSH) deficiency (10) seems to be a key factor for the severity of the SARS-CoV-2 disease. An antioxidant effect of H_2S may replenish GSH, thereby producing cytoprotective effects.
4. H_2S could attenuate the SARS-CoV-2-related ''cytokine storm'' due to the downregulation of the production of various pro-inflammatory mediators (11) and/or *via* the inhibition of leukocyte activation (12).
5. Endothelial dysfunction is a significant part of SARS-CoV-2 disease (13), and H_2S donors have been demonstrated to exert significant endothelium-protective effects in various experimental models (14). This point is especially pertinent, because the epidemiology of the SARS-CoV-2 disease clearly suggests a particular role of H_2S: the majority of patients needing mechanical ventilation and extra-pulmonary organ support are older and/or suffer from underlying chronic cardiovascular, metabolic, and/or pulmonary comorbidity. All these conditions are well known to be associated with impaired endogenous H_2S availability and/or reduced CSE expression (12).
6. In addition, H_2S was found to potentiate T-cell activation and regulate T_{reg}-cell-associated immune homeostasis, with the net effect being a stimulation of the immune response (15). Such effects may be beneficial in the context of stimulation of anti-SARS-CoV-2 immune responses.
7. Finally, recently, Renieris et al. (16) demonstrated in this journal that high baseline concentrations of reactive sulfur species and/or their lacking decrease over time were associated with worse outcome of SARS-CoV-2. While the absolute concentrations reported clearly have to be questioned (17), this observation nevertheless suggests investigating exogenous H_2S as a therapeutic approach.

It is self-evident that any potential therapeutic approach using H_2S donation raises the question of the route of application. Theoretically, two possible strategies could be considered: boosting endogenous H_2S formation, i.e., by supplementing upstream substrates and/or cofactors of the H_2S-producing enzymes (e.g., taurine, vitamin B6, α-keto-glutarate), or exogenous H_2S administration. The latter approach can either make direct use of the H_2S molecule itself, i.e., by inhaling gaseous H_2S, or use molecules that can ''release'' H_2S (Fig. 1). In this journal, Ali et al. (18) advocate a clinical trial to explore the use of inhaled H_2S for the management of SARS-CoV-2-related ARDS. Albeit inhaling gaseous H_2S has already been performed in small studies in healthy human volunteers (19), for several reasons, we strongly recommend NOT to use this approach in SARS-CoV-2 patients (20): due to its potential toxicity, its use requires special equipment and personnel for storage and handling, as well as close monitoring of the environmental and delivered concentrations to protect any bystander; it is well established that gaseous H_2S is an irritant of the airway mucosa; a direct comparison of inhaled H_2S and infusion of the H_2S-releasing salt Na_2S in murine ventilator-induced lung injury showed that in contrast to the protective effect of infusing Na_2S, inhaling H_2S dose-dependently had either no or even detrimental effects (21). Injection of the rapidly H_2S-''releasing'' salts (Na_2S, NaHS) results in initially high concentrations that subsequently rapidly disappear, and, in

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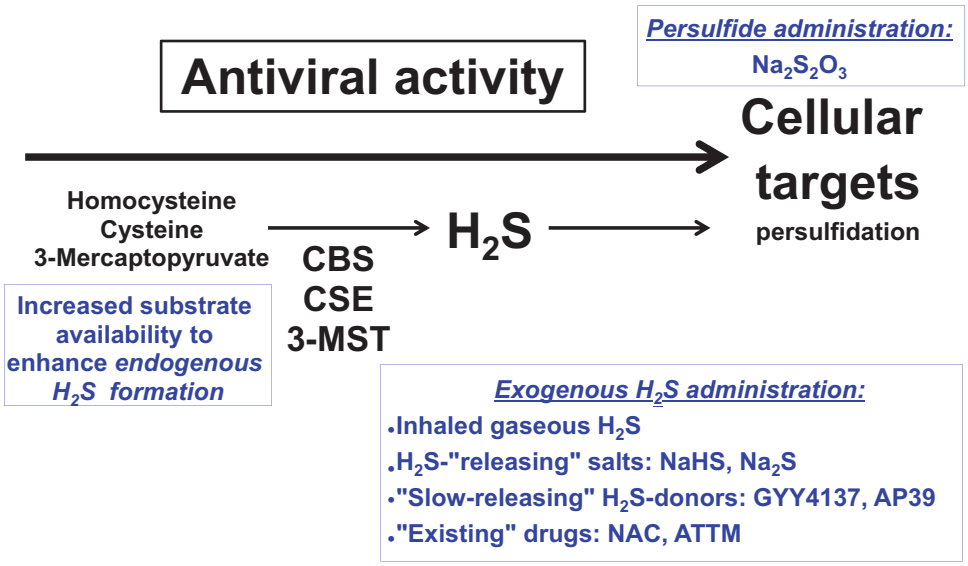

Fig. 1. **Pharmacological approaches to strengthen antiviral activity *via* increased H_2S availability in patients suffering from SARS-CoV-2 disease.** 3-MST indicates 3-mercaptopyruvate sulfurtransferase; ATTM, ammonium-tetrathiomolybdate; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; Na_2S, sodium sulfide; $Na_2S_2O_3$, sodium thiosulfate; NaHS, sodium hydrogen sulfide. Adapted from (32).

addition, may have adverse properties, e.g., induce pro- rather than anti-inflammatory effects (20). While at least under intensive care unit conditions, this inconvenient of bolus injection could theoretically be overcome by constant i.v., infusion, in clinically relevant large animal models, this approach had beneficial effects only within a narrow dose and time window (22). It is questionable as well, whether the recently developed "slow-releasing" H_2S donors, e.g., GYY4137 or the mitochondria-targeted compound AP39, will find their way into clinical practice: despite abundant promising experimental studies both *in vitro* and *in vivo*, the available data from fully resuscitated animal models showed either hardly any protective properties or even detrimental side effects (23, 24). Given the above-mentioned pitfalls of inhaling gaseous H_2S or infusing Na_2S-based i.v. solutions and the uncertainties of the newly developed compounds, interest has focused on the potential use of molecules, which are known sources of H_2S and are already recognized drugs for other indications. GSH replenishment can be achieved using its precursor *N*-acetyl-cysteine (NAC), which, moreover, would also potentially attenuate SARS-CoV-2-related "cytokine storm" (25) as well as allow for ACE2 inhibition (8). However, despite its promising pharmacological profile, in a single-center, double-blind, randomized, placebo-controlled trial in 135 patients, high-dose NAC (∼ 300 mg/kg over 20 h) did not beneficially affect the evolution of severe SARS-CoV-2 (26). Other potential candidates are ammonium-tetrathiomolybdate, which is recognized for the treatment of Wilson's disease, and sodium thiosulfate ($Na_2S_2O_3$), which is well established for the treatment of cyanide intoxication, *cis*-platinum overdose, and calciphyllaxis. Ammonium-tetrathiomolybdate (ATTM) showed promising results in rat hemorrhage and cerebral and myocardial ischemia/reperfusion (27). $Na_2S_2O_3$ not only was organ-protective in both murine endotoxin- and polymicrobial sepsis-induced acute lung injury (28), but, in particular, also improved lung mechanics and gas exchange in a clinically relevant, resuscitated long-term model of hemorrhage- and resuscitation in chronically comorbid swine characterized by coronary arterial CSE deficiency due to underlying ubiquitous atherosclerosis (29). It should be noted, however, that so far neither ATTM nor $Na_2S_2O_3$ have been investigated in patients with SARS-CoV-2.

In conclusion, there is sound evidence that H_2S bears the potential as a "defense against COVID-19" (4), in particular since currently there is no effective drug for the treatment of the disease. While inhalation of gaseous H_2S cannot be recommended so far, its administration (either *via* inhalation as aerosols and/or i.v. infusion (30)) using already recognized drugs that are well-established sources of H_2S in biological systems, warrants investigation in the clinical setting (31, 32).

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